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AMRL-TR-69-84

TOXIC HAZARDS RESEARCH UNIT
ANNUAL TECHNICAL REPORT: 1969

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SysteMed Corporation

SEPTEMBER 1969

JOINT NASA/USAF STUDY

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
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13. ABSTRACT

The activities of the Toxic Hazards Research Unit (THRU) for the period of June 1968 through May 1969 are reviewed in this report. The experimental research program was conducted concurrently with construction activities for additional facilities. The construction activities did not stop the research program but required cooperative scheduling of both activities to permit installation of utilities without interruption of experimentation. At scheduled periods, experiments were suspended to permit major corrective and preventive maintenance programs on both the ambient and altitude laboratory facilities. The Apollo materials toxicity screening tests have continued with no further evidence of toxicity exhibited by their gas-off products. A repeat eight-month study of the effects of a mixed gas (oxygen-nitrogen) atmosphere at 5 psia was completed with the only confirmed adverse effect being the depressed growth of albino rats. Experiments conducted in the ambient facility included studies on the effects of ethylene glycol vapor on rodents, studies on emergency exposure limits for monomethylhydrazine (MMH) and nitrogen trifluoride (NF_3), and preliminary acute toxicity experiments on oxygen difluoride (OF_2) and chlorine trifluoride (ClF_3).

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Medical Research						
Atmosphere Monitoring						
Space Cabin Toxicology						
Materials Testing						
Monomethylhydrazine						
Nitrogen Trifluoride						
Oxygen Difluoride						
Chlorine Trifluoride						

FOREWORD

This is the fifth annual report of activities of the Toxic Hazards Research Unit and concerns work performed by SysteMed Corporation on behalf of the Air Force under Contract No. F33615-67-C-1025. This constitutes the final report under the subject contract and describes the accomplishments of the THRU from June 1968 through May 1969.

The contract for operation of the laboratory was initiated in 1963 under Project 6302 "Toxic Hazards of Propellants and Materials," Task No. 02 "Toxicology," Work Unit No. 008. K. C. Back, Ph.D., Chief of the Toxicology Branch, is the technical contract monitor for the 6570th Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D., of SysteMed Corporation, served as principal investigator and Laboratory Director for the THRU. Acknowledgement is made to C. E. Johnson, C. C. Haun, G. L. Fogle and J. H. Archibald for their significant contributions and assistance in the preparation of this report. The National Aeronautics and Space Administration provided support for Apollo Materials Screening Program.

This report is designated as SysteMed Corporation Report No. W-69006.

This technical report has been reviewed and approved.

C. H. KRATOCHVIL, Colonel, USAF, MC
Commander
Aerospace Medical Research Laboratory

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SECTION I

INTRODUCTION

The Toxic Hazards Research Unit (THRU) is an interdisciplinary inhalation toxicology team which conducts toxicologic investigations of potentially hazardous materials for the Air Force. These investigations are conducted by SysteMed Corporation personnel and supported by the Veterinary Medical Division and Toxic Hazards Division of the Aerospace Medical Research Laboratory. The nature of the research program is based upon the needs and requirements of the Air Force to characterize the acute and chronic toxic hazards of materials used by military personnel and to which they may be unavoidably exposed. The scope of the research program also includes investigations of space flight environments with particular reference to the effects of continuous exposure to trace contaminants. The ultimate purpose of the investigations conducted by the THRU is to define the health risk of contaminant exposures for man and to establish safe environmental standards for both space flight and normal military working conditions.

The support services provided by the Air Force include procurement of laboratory animals, veterinary medical care, clinical and pathological examinations of animal tissues, computer analyses and concurrent basic pharmacologic investigations on mode of action and metabolism.

The Toxic Hazards Laboratory, in which the toxicologic investigations are conducted, consists of an ambient laboratory with standard inhalation exposure chambers for both large and small animals, a preconditioning laboratory in which new animals are acclimatized to experimental conditions, and two altitude laboratories each of which has four specially designed altitude chambers (designated hereafter as Thomas Domes). The Thomas Domes are utilized for exposing animals to contaminants under conditions which simulate space flight conditions as closely as possible, with the exception of radiation and weightlessness, and have a capability of providing atmospheric compositions of 100% oxygen or varying mixtures of oxygen and nitrogen at absolute pressures ranging from ambient to as low as 5 psia (1/3 atmosphere). Detailed discussions of the design and operation of the THRU facilities are published in references 4, 7, 8, 10 and 13.

This report summarizes the research accomplishments of the THRU from June 1968 through May 1969 and includes various facility and design modifications made since the last annual report (reference 10). During the past year the experimental research program was conducted concurrently with a military construction project (MCP) for additional research facilities. The construction activities, which were nearing completion at the end of the current

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report period, did not stop the research program but required cooperation in scheduling of both activities to prevent loss of experimentation and permit the installation of electrical, water and steam services required for the construction project. The MCP included the second altitude laboratory previously mentioned with its four new Thomas Domes. These new domes, in which experiments are scheduled to begin during June 1969, are equipped with a special interconnecting airlock that permits passage from dome to dome without multiple depressurizations and repressurizations of personnel or experimental animals. The interconnecting airlock will also serve as a surgical suite for conducting physiological function measurements under the selected environmental exposure conditions.

SECTION II

FACILITIES

GENERAL

The primary mission of the THRU is to conduct applied toxicologic investigation on materials of interest to the Air Force. The operation of a research laboratory for this purpose requires a variety of supporting activities in addition to those provided by the Air Force as described earlier. The analytical chemical services and data analysis which were integral parts of specific experimental programs will be discussed in a later section of this report. Various activities of the THRU program are not of significant magnitude to merit separate technical reports and will be reviewed under the general heading of "Facilities." The activities and services include personnel training programs, engineering modifications of the research facilities, development of computer programs and special projects in analytical chemistry. Although a number of engineering modifications in the last annual report were completed during the current report period, only these alterations made in the interim period are discussed in detail.

During the past year, the standard operating procedures (SOP's) of the THRU were thoroughly reviewed and revised in accordance with engineering modifications and increased knowledge of the operating characteristics of the Thomas Domes. One of these changes involved the extension of the oxygen prebreathing period prior to dome entry when the work to be performed by dome entrants was expected to be very strenuous such as the catching and weighing of monkeys. The revised SOP's are considered suitable for initial operation of the new altitude laboratory until sufficient experience has been gained with those components unique to the new systems.

The maintenance manuals developed for THRU operations have become outdated by engineering improvements to the ambient and altitude laboratories made throughout the last three years. These manuals are currently undergoing revisions which will not be considered complete until the unique systems of the new Thomas Domes are included. This effort should be complete early in the next report period.

BIOSTATISTICAL SERVICES

The use of computer analysis for data generated in the research program has increased steadily over the past year. Several additional computer programs of the BMD series developed by the School of Medicine at UCLA were modified for use with THRU experimental data. The new programs placed in

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service perform sorting operations, frequency counts, data graphs and histogram compilations. These features can also be used for THRU activities other than biological data analysis and are currently being worked up for use in property control and in maintenance material ordering. One effect of the increased use of computer programs for data analysis was a revision in the method of recording and handling of the experimental data. This change has resulted in a standard approach to units of measurements made in the THRU laboratory.

The method of selection of rodents for use in experiments was changed to a completely random selection technique using a computer program of random numbers. The random selection is made, however, only from those animals approved for use after the usual quarantine period.

In previous years large animals were examined clinically and the results of baseline determinations from a battery of hematologic and clinical chemistry parameters were used in determining their suitability for use in experimentation. The standards used for evaluation of health status were based on the large volumes of historical data that had been collected over previous years. The historical data were subjected to a critical review and found not representative of current animals. Animals currently used were found to belong to a different population group, and the historical data also suffered from subsequent changes in methods of chemical analysis of blood and in the laboratory instrumentation used for those measurements. In an effort to improve the system of large animal selection and to evaluate blood chemistry measurements made during experimental exposure of the animals, a new system has been placed in use. A new "standard" control group consisting of all dogs and monkeys awaiting use in experiments plus those currently in use as experimental controls are tested biweekly and the results of these determinations are pooled for six successive periods for calculation of mean and standard deviation. With each successive addition of new data the earliest sample data are eliminated. The 95% confidence limits of the mean of the "standard" control group are calculated on the basis of the number of animals in standard experimental control groups serving as the appropriate number of degrees of freedom. These values are then plotted as moving limits for evaluation of the clinical hematology and chemistry measurements made on exposed animals in each experimental group. This technique provides a realistic assessment of exposure effects while eliminating confusion resulting from variations of seasonal or analytical nature. It also provides a visual presentation of control and exposure data on a continuing and current basis for the management of the experimental program.

PERSONNEL TRAINING PROGRAMS

In preparation for the use of the new altitude facility, additional chamber technicians and facility engineering technicians were employed and placed in training. This training program was also provided for new Air Force chamber

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operators who were assigned to the Toxic Hazards Division in the past year. Experienced chamber technicians attended the training sessions to refresh their job skills and to learn the operation of new or modified equipment resulting from engineering changes.

The training of facility engineering technicians consists of a series of lectures and demonstrations on each of the various control systems and subsystems required for operation of the Thomas Domes. The technicians are provided a loose-leaf book containing specific emergency and preventive maintenance procedures with diagrammatic displays of each system. The specific sections of the book are presented to the technicians when the lectures on that system are made.

The size and complexity of the Toxic Hazards Research Laboratory are sufficiently large to require some specialization within the Facility Engineering Department and accordingly, the department has been divided into a mechanical services section and an electrical services section. Concurrent with the general training program on all systems that the Facility Engineering technicians receive, they are given specialized on-the-job training in the sections to which they are assigned.

The chamber technician training program consisted of a series of didactic lectures combined with demonstrations and on-the-job experience. The job training involved the four primary functions of Thomas Dome operation, shift operator-Observer "A," dome entrant and safety Observers "B" and "C." Special training in animal care and experimental techniques was also provided which involved the use of the Purina "Laboratory Animal Care" course along with film presentations on this subject.

A closed circuit television system, obtained in the preceding year, was used extensively in the program. Video tape visual aids were prepared on the following subjects:

1. Rescue of an Incapacitated Dome Entrant
2. Thomas Dome Fire Control System Operation
3. Fire in the Dome
4. Fire in the Airlock
5. Liquid Oxygen System Failure
6. Complete Power Failure
7. Air Compressor Failure
8. Vacuum Pump Failure
9. Liquid Oxygen System Operation
10. Clinical Chemistry and Venipuncture Techniques for Animals

In addition to the training programs conducted within the THRU several members of the staff attended special training courses. The Miami Valley Safety Training Conference provided specific lectures of interest to the THRU program and included "Hazardous Materials Handling," "Use of High Pressure Gases," and "Operation of Self-Contained Breathing Apparatus." A 2-day special training course on mass spectroscopy sponsored by the American Chemical Society and a Bendix Mass Spectrometer Training School were attended by two chemists.

ANALYTICAL CHEMISTRY PROGRAMS

The principal functions of the Analytical Chemistry Department of the THRU are to develop methods of analyses for trace atmospheric concentrations of materials to be used in toxicity investigations and to perform the routine task of monitoring animal exposure chamber contaminant concentrations during the conduct of experiments. Some analytical projects, of equal importance in the overall objectives of the THRU mission, do not directly relate to toxicological research in progress. These projects, including contaminant pyrolysis product studies and methods development for related Air Force toxicity experiments are the subject of this portion of the annual report.

Fluorel Pyrolysis

A sample of Fluorel, used as a sole material for astronauts' boots, was examined for potential hazardous constituents when pyrolyzed.

A mass spectrometric study was made of the gases evolved from Fluorel after pyrolysis in an oxygen atmosphere, at temperatures up to 350 C. Fluorel is a copolymer of vinylidene fluoride, $CH_2 = CF_2$, and hexafluoropropylene $CF_3CF = CF_2$. The repeating unit is:

$$\left[\begin{array}{c} -CF_2 - CH_2 - CF \quad - CF_2 - \\ CF_3 \end{array} \right]_n$$
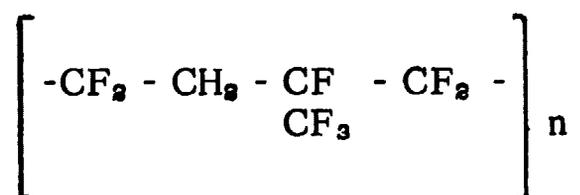
Samples of approximately 0.1 grams were weighed and placed into a stainless steel high pressure sample cylinder of 75 ml volume. The cylinder was then successively evacuated and pressurized with oxygen to obtain a 100% oxygen environment. The cylinder was heated to 350 C and after approximately 15 minutes the pyrolysis was assumed to be complete. The cylinder was then cooled and connected to the batch inlet system of the mass spectrometer. After meaningful spectra had been recorded, the pyrolysis procedure was repeated. Seven pyrolyses were carried out in the steel cylinder and a mass spectrum recorded of each. The results were all similar, with carbon dioxide being

TABLE I

A Comparison of Pyrolyzates from Fluorel and Teflon

Mass No.	Ion$^+$	Teflon Relative Abundance*	Fluorel Relative Abundance
12	C	0.10	0.18
14	N	5.26	3.00
16	O	1.74	2.00
19	F	0.02	0.01
20	HF	0.16	0.10
23.5	COF	0.01	N.D.
28	N_2	100.00	100.00
31	CF	0.17	0.30
32	O_2	27.18	23.00
40	A	2.40	2.00
44	CO_2	1.72	17.00
47	COF	1.90	0.10
50	CF_2	0.11	0.18
66	COF_2	1.16	N.D.
69	CF_3	0.62	1.80
81	C_2F_3	0.003	1.00
85	SiF_3	0.00	0.50

*(reference 1)

the most abundant product formed in each case. Since "Fluorel" is similar to polytetrafluoroethylene (Teflon), some similarity in the pyrolysis products can be expected. In Table I, the mass spectrum of the pyrolysis products of Teflon is presented along with typical results obtained for Fluorel in this study.

Fluorel did not generate COF_2 on pyrolysis and as noted before, CO_2 was the most abundant product formed. Only a minor amount of small fluorocarbon fragments are found in the pyrolyzate spectrum. Since hydrogen and fluorine are present in the Fluorel structure, some HF might be expected to result from its pyrolysis. However, no HF fragments were detected in the mass spectrum of pyrolysis products. Samples of Fluorel were then pyrolyzed in a glass tube under essentially similar conditions employed with the steel cylinder. The results from the glass tube differ from the results from the steel cylinder as illustrated in Table II.

TABLE II

Comparison of Fluorel Pyrolyzates

Formed in Glass and Steel Reaction Vessels

Mass No.	Ion+	Relative Abundance in a Glass Vessel	Relative Abundance in a Steel Vessel
69	CF_3	1.7	1.8
85	SiF_3	8.1	0.5

Hydrogen fluoride is apparently formed during the pyrolysis of Fluorel and reacts with the container to form SiF_4 in glass, and essentially nonvolatile fluorides in stainless steel. Fluorel did not undergo spontaneous and complete combustion, such as that seen with carboxy nitroso rubber (reference 9), and most of the polymer was still present after treatment. This material appears to be as safe as Teflon under pyrolysis conditions.

Analysis of Thomas Dome Atmospheres

The enclosure of animals in an inhalation exposure chamber for toxicity investigations results in the production of a characteristic odor which is not easily eliminated without the use of prohibitively high air exchange rates. The odors are most readily noticed in exposure chambers such as the Thomas Domes where research personnel enter the chamber to make biological measurements on the animals and to provide routine animal care. These odors appear to be

metabolic waste products of the animals, possibly combined with oxidation products of body wastes. Thus, the question has been posed, "Does this noticeable odor constitute a physiologic stress upon the experimental animal and what is the nature of the contaminant or contaminants causing it?" Although no significant differences have been found between animal room controls and animals housed in the Thomas Dome, the question still has merit in that the dome environment is not completely defined.

A series of experiments were initiated to define the composition of the "dome odor." The initial efforts were directed toward finding a satisfactory method for collection and concentration of the unknown dome contaminants for subsequent analysis. A significant problem in sample collection has resulted from the amount of water vapor present in the chamber effluent gas.

As of the end of the current report period samples have been collected in gas chromatographic columns using column packings of Poropak Q, chromosorb 103 and activated charcoal. A condensate trap was placed in the sampling line ahead of the chromatographic column and the water collected by this method also subjected to analysis. Additional samples were collected within the Thomas Domes on a shallow bed of activated charcoal from which the contaminants were subsequently distilled and collected for analysis.

Fifteen individual gas chromatographic peaks in the samples collected in the columns have been detected with the aid of a flame ionization detector. Of these 15 peaks, only methane has definitely been identified although work is continuing on the identification of other constituents. Ammonia has been identified in the water condensed from the chamber effluent by means of a colorimetric procedure utilizing Nessler's Reagent. A gas sample of dome effluent was absorbed in 0.1N sulfuric acid and back titrated potentiometrically to provide a measure of the concentration of volatile alkaline materials in the atmosphere. A concentration of 0.9 mg/M^3 calculated as ammonia was determined from replicate samples.

These experiments to identify trace contaminants of animal origin in the Thomas Dome will continue until we can provide a reasonably accurate profile of the characteristic odor observed.

Bromotrifluoromethane
_____

A gas chromatographic method for monitoring bromotrifluoromethane (Halon 1301) was developed for short-term animal exposures and incorporated into a special primate cage equipped for negative avoidance performance measurements. The atmospheric concentrations in the exposure chamber were established for a CBrF$_3$ range of 20-80%. A Porapak Q column was operated at a temperature of 80 C for the separation of CBrF$_3$ from air using a helium carrier gas.

Blood Analysis for Carbon Monoxide
_____

A broad series of experiments to define the biological effects of carbon monoxide were initiated during the past year and a biochemical method for determining CO exposure was needed for correlation with measured biological responses. The carboxyhemoglobin procedure of Dominguez, et al (reference 2) has been modified for our use in the blood analysis of animals exposed to carbon monoxide. The basic procedure is the same, CO release from sample and saturated blood with ferricyanide, then gas chromatographic analysis. Modifications in the procedure have been made to use our facilities in the most accurate and efficient manner.

Materials

Aerograph Gas Chromatograph A-90-P.
> Four foot, 5A molecular sieve column
> Column temperature 85 ± 3 C
> Helium flow rate 77 ml/minute
> Nickel hot wire thermal conductivity detector at 250 milliamps.
> Burette, 50 ml
> Helium and carbon monoxide gas cylinders
> Impinger flask, 30 ml
> Pipettes, 5 ml
> Serum stoppers for flasks
> Syringes, 1, 5, 10 and 30 ml
> Vortex mixer

Reagents

Acetate buffer - dissolve 15 ml glacial acetic acid in 85 ml distilled water, add 42.2 g anhydrous sodium acetate and dissolve.

Potassium ferricyanide (prepared daily) - dissolve 48 g potassium ferricyanide and 1 g saponin in 160 ml warm distilled water. Cool and dilute to 200 ml with acetate buffer.

Petrolatum oil

Octanol - 2

Method

Heat the gas chromatograph daily to 240 C to flush out any water in the column, then cool to 85 C before use. Draw the blood, inject

it into a rubber stoppered test tube containing heparin, and store in a refrigerator until use. When ready for use, allow the blood to warm to room temperature, mix by shaking and pipet 4 ml into a beaker after the foam settles. Dilute with 36 ml distilled water. Mix thoroughly and draw off a 5 ml sample with a syringe and inject into a serum stoppered, helium flushed 30 ml graduated impinger flask. Flush the impinger flask by means of a needle inlet through the serum stopper from a helium tank for about 15 minutes. The exit is a needle through the serum stopper to a bubbler hose. Remove the helium flush inlet but leave the exit bubbler in the stoppered flask until the 10 ml potassium ferricyanide solution is injected. Swirl the flask on the mixer for three minutes, taking care to make as little foam as possible since the foam captures some of the evolving carbon monoxide. Introduce 4 drops of octanol into the flask with a syringe to defoam. Allow the flask to sit for 5 minutes before sampling. Withdraw 2 ml of gas and flush into the air, then withdraw over 10 ml of gas from the tube as distilled water is gravity fed into the flask to replace the gas withdrawn. Ten ml of this gas is injected directly into the gas chromatograph. Run duplicate samples. For CO saturation, sample the diluted blood by drawing 8-9 ml into a 30 ml oiled glass syringe. Further, draw 21-22 ml of pure carbon monoxide and 0.2 ml of 2-octanol (antifoam), cap with a serum stopper and shake well for 10 minutes. Eject the excess gaseous carbon monoxide and allow to stand for 5 minutes. Withdraw 5 ml of the saturated blood using a 5 ml syringe and inject into a helium flushed impinger flask. Swirl the flask for 1 minute to release any trapped gas; then helium flush again for about 5 minutes. Flushing is complete when a 10 ml gas sample from the tube shows no carbon monoxide peak on the gas chromatograph. Add potassium ferricyanide and proceed as for the unsaturated sample.

Calculations

$$\text{Blood CO saturation, percent} = \frac{\text{Peak height of sample}}{\text{Peak height of saturated blood}} \times 100$$

General

Calibration experiments conducted by injecting different volumes of pure carbon monoxide into the gas chromatograph demonstrated that the peak height increased linearly with volume as shown in figure 1. When different volumes of CO saturated dog and rhesus monkey blood were analyzed, linear calibration curves were obtained (figures 2 and 3) indicating that quantitative release of CO from the blood was attained.

The precision of the carboxyhemoglobin procedure has been estimated by analyzing a series of replicate samples. The coefficient of variation of carboxyhemoglobin values in saturated blood is 1.9 and 3.4 in blood samples of animals exposed to low CO air concentrations.

THE EFFECT OF VARYING C O VOLUMES
ON GAS CHROMATOGRAPHIC RESPONSE
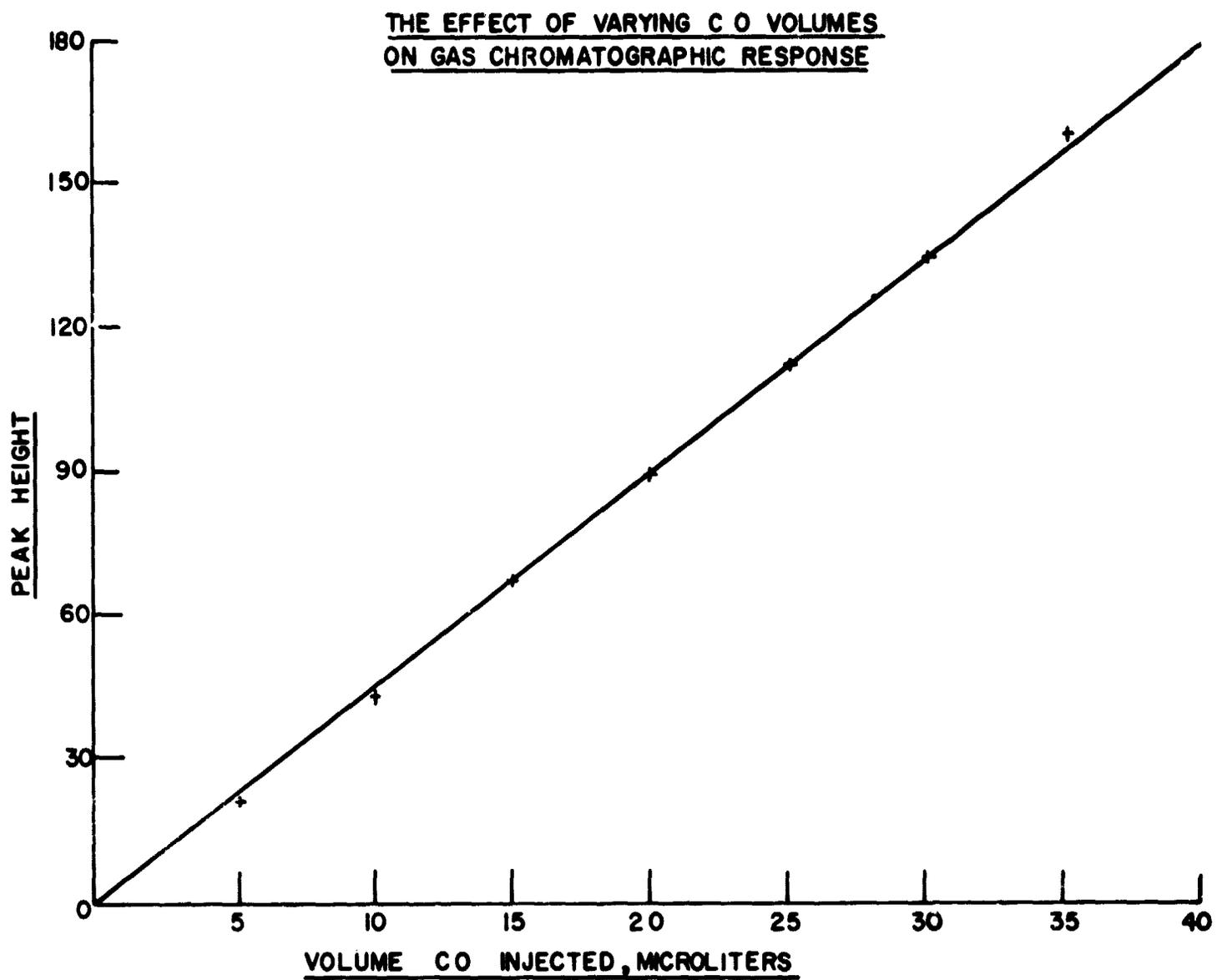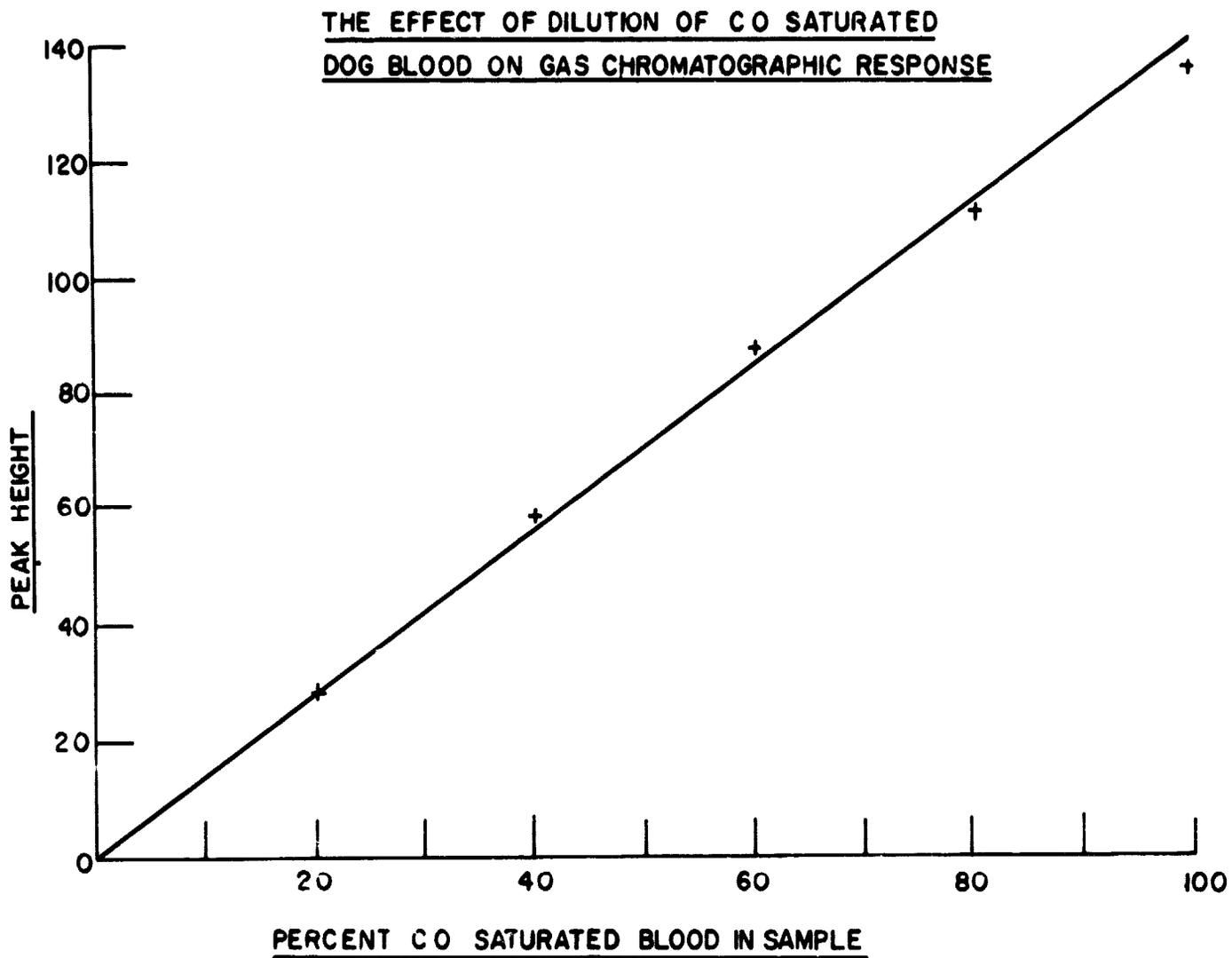
Figure 1

12

THE EFFECT OF DILUTION OF C O SATURATED DOG BLOOD ON GAS CHROMATOGRAPHIC RESPONSE

PEAK HEIGHT

PERCENT C O SATURATED BLOOD IN SAMPLE

Figure 2

13

Figure 3

14
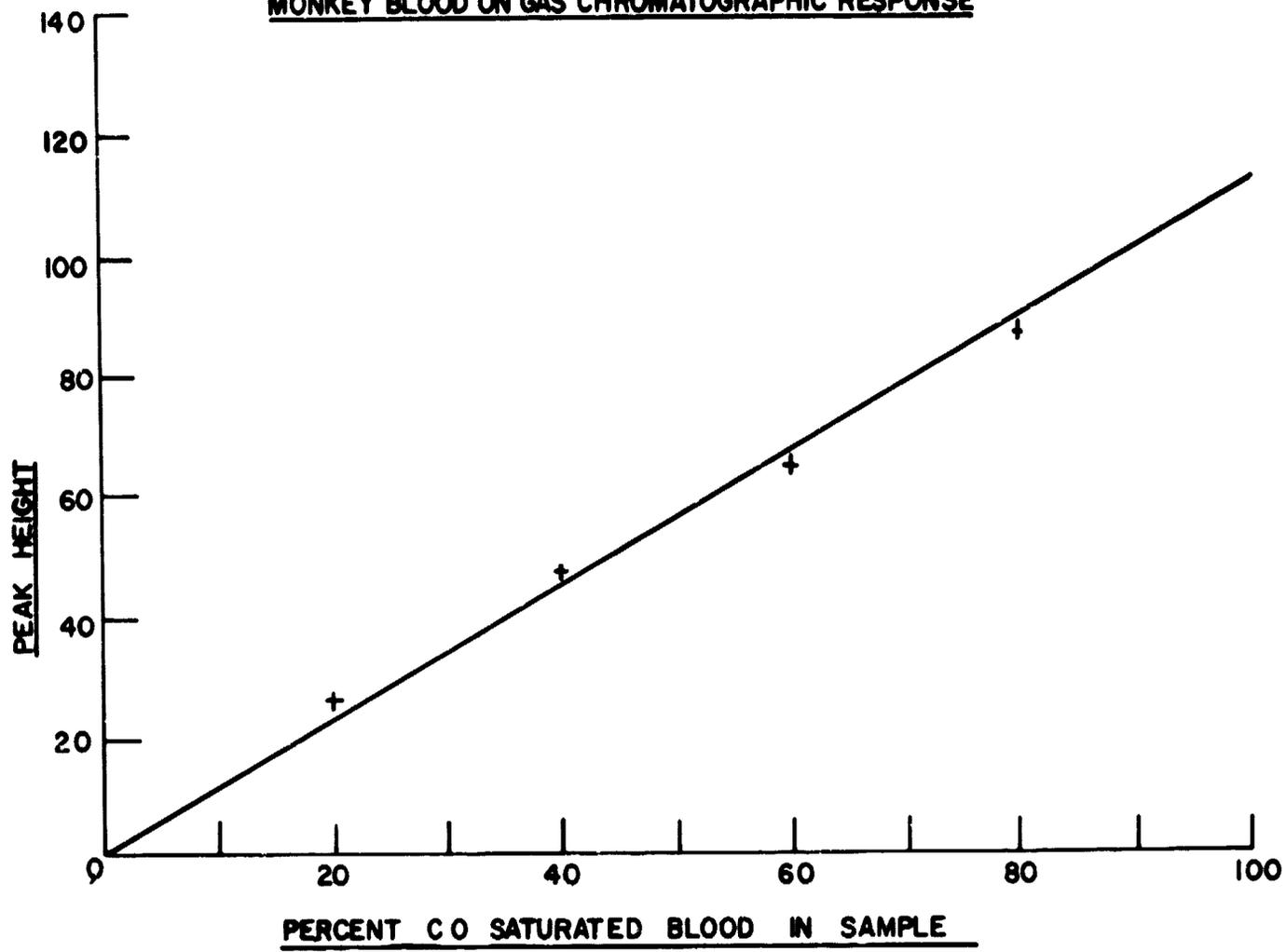
Chlorine Trifluoride

After characterizing the toxicity of NF_3, the first in a series of fluorinated oxidizers of interest to the Air Force, work was begun on the investigation of the toxicological properties of ClF_3. In order to support the toxicological experimentation, methods for the analysis of ClF_3 concentrations over the range of 0-1000 ppm were required. Because of the highly reactive nature of the compound, special techniques were necessary for the quantitative analysis of this compound.

The Billion-Aire analyzer was selected as having the most potential for successful analysis of ClF_3 because of its simplicity, speed of response, and continuous analysis capability. Standard concentrations of ClF_3 were made by drawing up the pure gas into a teflon syringe and injecting it into nitrogen filled teflon bags. After mixing, the bag atmosphere was attached to the Billion-Aire sample line and analyzed. The instrument has such high sensitivity that the method requires the atmosphere containing the ClF_3 to be diluted to 50 ppm or lower by introducing dry air at the required flow rate. The ClF_3 then reacts directly with dimethylamine reagent to form an aerosol whose concentration (and indirectly that of the ClF_3) is measured by the electron capture detector.

Although published reports (reference 12) indicated that a stainless steel system, such as had been constructed for the introduction of samples into the Billion-Aire, could be made stable to ClF_3 by passivation with the pure gas, it became evident that the analytical measurements were affected by the passivation procedure. Passivation of the metal surfaces by this material appears to be a function of the concentration delivered, and requires a different set of conditions for each concentration tested.

The analyzer conditions and precautions necessary because of the reactive nature of ClF_3 were defined and calibration curves over the range 0-1000 ppm constructed. The ClF_3 method was then written as a standard procedure for use in toxicological investigations which are presently being conducted.

Engineering Programs

The principal emphasis of the Engineering Department has been placed upon preventive maintenance during the past year. The ambient laboratory equipment having had almost 6 years service and the altitude laboratory 5 years of operation, any components of the various operational systems begin to require more service and occasional replacement. In some cases modifications were made at the time replacement became necessary. These modifications were made based on experience with the equipment, technological advances, and upon current experimental requirements.

A significant part of the engineering activity was involved in the preparation for use of the 4 new Thomas Domes. Animal caging and watering systems had to be designed and fabricated or purchased, communication systems installed and many accessory systems made operational. These activities were not complete at the end of the report period due to delays in completion of the construction project. Those modifications, projects of importance to the operation of the inhalation toxicity exposure system, will be discussed separately.

Dome Exhaust Rings

The portions of the vacuum exhaust systems located within the domes were modified by replacement of the original floor supported rectangular exhaust rings with 4-inch diameter round rings which are supported from the side walls of the dome base. The new configuration provided an increase in dome space, improved safety and provided for easier cleaning of the exhaust ring area. The rings were installed in four sections with bands to permit easy removal for required maintenance. The support of the dome water flush rings was also changed from the dome floor to the side wall of the dome base.

Rodent Cage Supports

The rodent cage system in the Thomas Domes was originally supported from the dome top. They were difficult to service in this location and occasionally broke loose. Therefore, a support system firmly attached to the dome base was designed to hold the cages firmly in a more favorable location for service. Angular supports, designed to parallel the angle of the dome top, were constructed and bolted to supports welded to the dome base. The angular supports can, therefore, be easily removed for any purpose. The system consists of two tubing rings located on angle brackets bolted to the vertical supports. The inner ring serves only to support the cages while the outer ring is used both for this purpose and also as the automatic watering system supply line. Cages may either rest on or hang from the rings leading to increased cage capacity. Supporting the cage system from the dome base eliminates the requirement of supplying water to the dome top through a quick disconnect system. This reduces the potential hazard involved in raising the dome top, particularly in emergency situations. The improved accessibility of the cages has significantly decreased the time required in animal feeding and cleaning under altitude conditions.

Sequential Sampling of Dome Atmosphere for CO Analysis

Since experiments using CO as a contaminant were scheduled for operation in a number of domes simultaneously, it was decided to use the existing dome CO_2 analyzer as a sequential sampler for a single CO analyzer. The CO monitoring instrument was connected to the exhaust vent of the CO_2 analyzer

and the sampling period for each dome in the sequence was increased from 1 minute to 5 minutes. This was sufficient time for complete flushing of previous samples and the attainment of a constant concentration in the CO analyzer. The CO_2 system not only samples the domes in sequence but also switches the air pressure signal from the CO analyzer to the correct panel recorder.

Dome Flight Tape Recorder

A voice actuated tape recorder was installed at the main control panel for recording communications between the observers and dome entrants during a dome flight. In the event of any accident, this tape will serve as a valuable first hand record of what occurred. The tape size is sufficient for recording daily entries into all four domes. A loudspeaker has also been added to the system to increase the number of communicants without overloading the system.

Vacuum Pump Cooling Water Drain

The original dome vacuum pump cooling water drain lines were manufactured of galvanized sheet metal which presented corrosion and maintenance problems. These were replaced by 4-inch stainless steel pipe. Guards were also installed at each muffler output to reduce excessive splashing and spraying.

Airlock Oxygen Concentration Measurement

In accordance with the revised Thomas Dome SOP's the airlock containing a dome entrant must be purged with oxygen at a pressure below 400 mm Hg to minimize potential fire hazards. Under these conditions the operation of a manual oxygen sensor is difficult since a calculation involving the actual measurement pressure must be made to determine the corrected oxygen concentration and to decide when the purge is completed. With a view toward correcting this problem and also to achieve continuous monitoring of the airlock oxygen content, a system for remote analysis of airlock oxygen concentration was designed. The system provides continuous monitoring of the oxygen concentration in the airlock being used by sampling the airlock environment with a paramagnetic O_2 sensor through a selector switch operated by Observer B from a basement location. A display of the measured oxygen concentration is available at two positions; the Observer B station in the basement and the main control panel. The system has been in satisfactory operation for four months.

UV Sensor Tester

With the installation of the UV flame detector and water deluge system in the Thomas Domes, sensitive, fast reacting fire protection units became operative which significantly reduced the hazard of fire in the domes. The manufacturer's recommended method of testing whether the sensors were in

operating condition was to hold a flame in the sensor field while the system was in the "Test" condition. This procedure was not feasible during an experimental study, and an alternative method of routine testing had to be devised. The obvious solution appeared to be a UV source which could be taken into the dome and used to activate the sensors. Commercially available UV sources suffered from serious drawbacks, however. They were either too heavy to manipulate in the dome or required a sufficiently high voltage to constitute fire hazards themselves. The solution was achieved by design and construction of a battery system to power a 4 watt UV bulb. Two 15 volt batteries were used in series with a resistance. Thus, total voltage available never exceeds 30 volts, and this requirement decreases to less than 20 volts under load. These voltage ratings are considered safe for use in oxygen rich environments. A quartz window covers the bulb to allow maximum UV transmission. Through the use of this UV source on routine safety checks, a number of nonfunctioning detectors have been discovered and replaced.

Emergency Alarm System

The main emergency alarm system was modified to provide increased capability for the new domes and the display stations were relocated consistent with the construction modifications that took place. Interconnections were made between the existing and new alarm systems.

Animal Caging and Watering

Dog pens were fabricated for the new domes. These pens constituted a major redesign from the existing cages. To facilitate dome cleaning and ease of animal handling, the cages were suspended from the side of the dome base, eliminating the need for individual support legs.

Rodent cages are supported by racks in the same manner that proved highly satisfactory in the old domes. Approximately 25 cages can be placed in each dome. This number of cages is sufficient for exposing 100 rats or 250 mice or some intermediate combination of these species.

Animal drinking water pressure regulation controls were installed adjacent to the new domes. Two water regulators are provided at each dome to provide a backup water supply in case of regulator failure and to permit their removal for periodic maintenance.

Relative Humidity Control

Final construction and installation of the relative humidity probe holders in the new domes was completed. A power supply for the wet-bulb blower motors was also installed. Preliminary checkout indicates that the system is functioning according to design specifications.

Ambient Laboratory Modifications

Since the ambient laboratory began operation over five years ago, it has been used extensively for experiments using reactive substances such as pyrolysis products of fire extinguishant materials and monomethylhydrazine in addition to materials which were relatively inert. During this time, a number of modifications were suggested which would make the ambient exposure facility more versatile. Also during this period the original duct work had undergone corrosive changes that made replacement necessary. Furthermore, while planning for investigations using oxidizer materials such as ClF_3 and CLF_5, it became evident that certain modifications were necessary to conduct these experiments with maximum safety for the toxicologists and technicians and to insure results that could be interpreted unambiguously.

Specific alterations were designed for improved performance in the following areas:

a. Elimination of Leaks

All exhaust lines in the ambient laboratory were replaced with welded stainless steel pipe. They were also repositioned as shown in figure 4 to provide a more direct and leak free route to the contaminant scrubber towers and then to the roof exhausts.

The exposure chamber exhaust blowers were originally installed between the chamber and the water scrubber towers and constituted a potential source of contaminant leakage. Consequently, a false ceiling had been installed below and around these blowers to isolate them from the general laboratory area. The enclosure in which the blowers were located was provided with a negative pressure exhaust system to assure no back contamination of the laboratory.

In the redesign of the chamber ventilation system, these blowers were relocated to a penthouse located on the roof of Building 79 directly above the ambient laboratory as shown in figure 5. They became the last point in the exhaust system except for a short discharge stack above the penthouse. In this manner the entire ambient facility exhaust system within the laboratory area operates under negative pressure, which prevents contamination of the work areas.

b. Increased Flow Rates

The original ambient exposure chamber air supply blowers were removed from the chamber air conditioning system and the ducting was redesigned to permit installation of an increased capacity blower. Manual switching of this blower was modified to utilize a standard one horsepower motor starter with integral overload protection.

ROCHESTER EXPOSURE
CHAMBERS (2)

LONGLEY EXPOSURE
CHAMBERS (2)

EXHAUST AIR FLOW UP
TO BLOWERS ON ROOF (4)

CHAMBER EXHAUST
SCRUBBERS (4)

ELECTROSTATIC
PRECIPITATOR

C

A

D

B

WATER
SUPPLY

LAMINAR FLOW
ELEMENT

INPUT BLOWER

INPUT AIR CONDITIONING SYSTEM
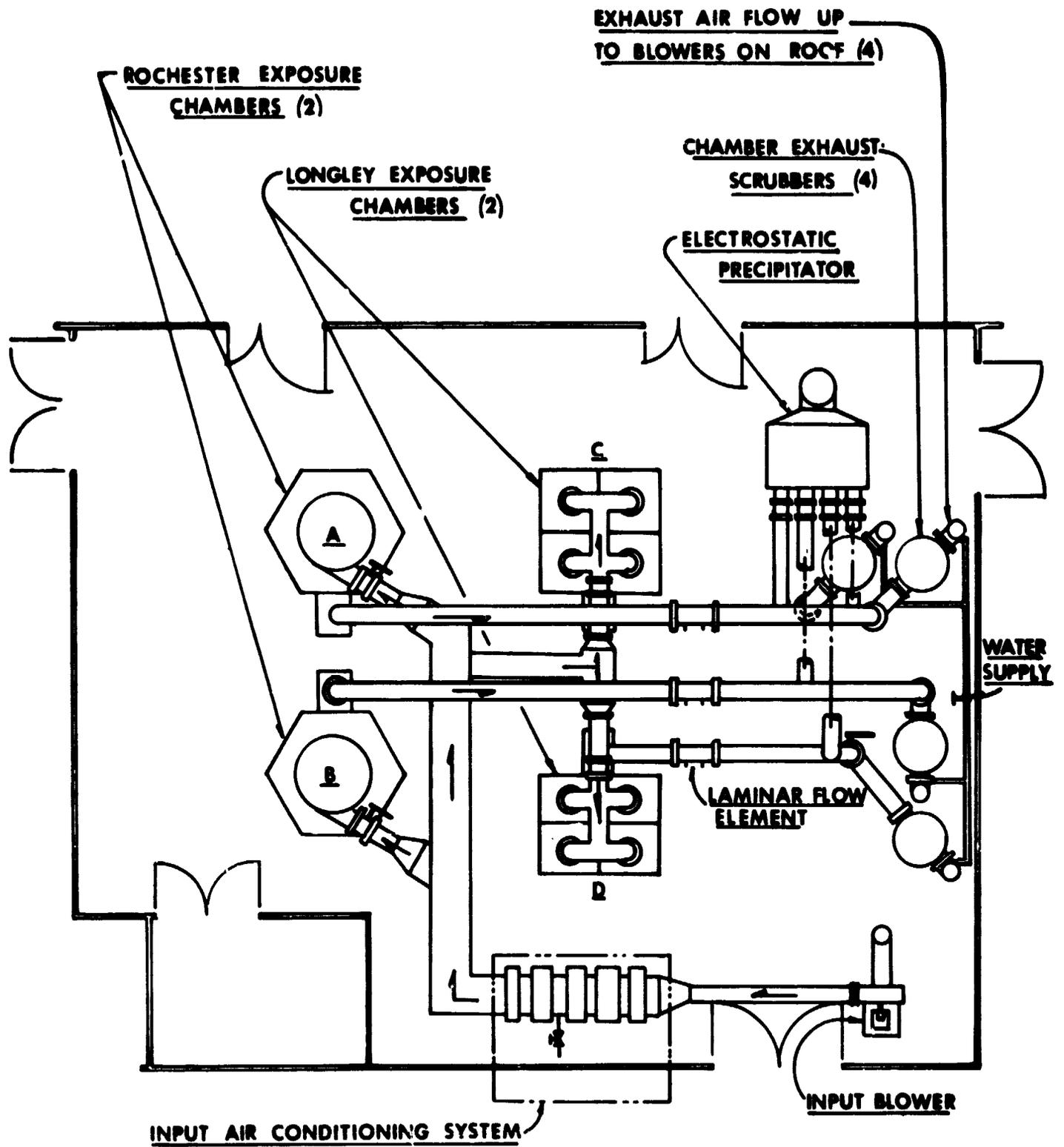
AMBIENT EXPOSURE CHAMBER
VENTILATION SYSTEM
PLAN VIEW

Figure 4

20
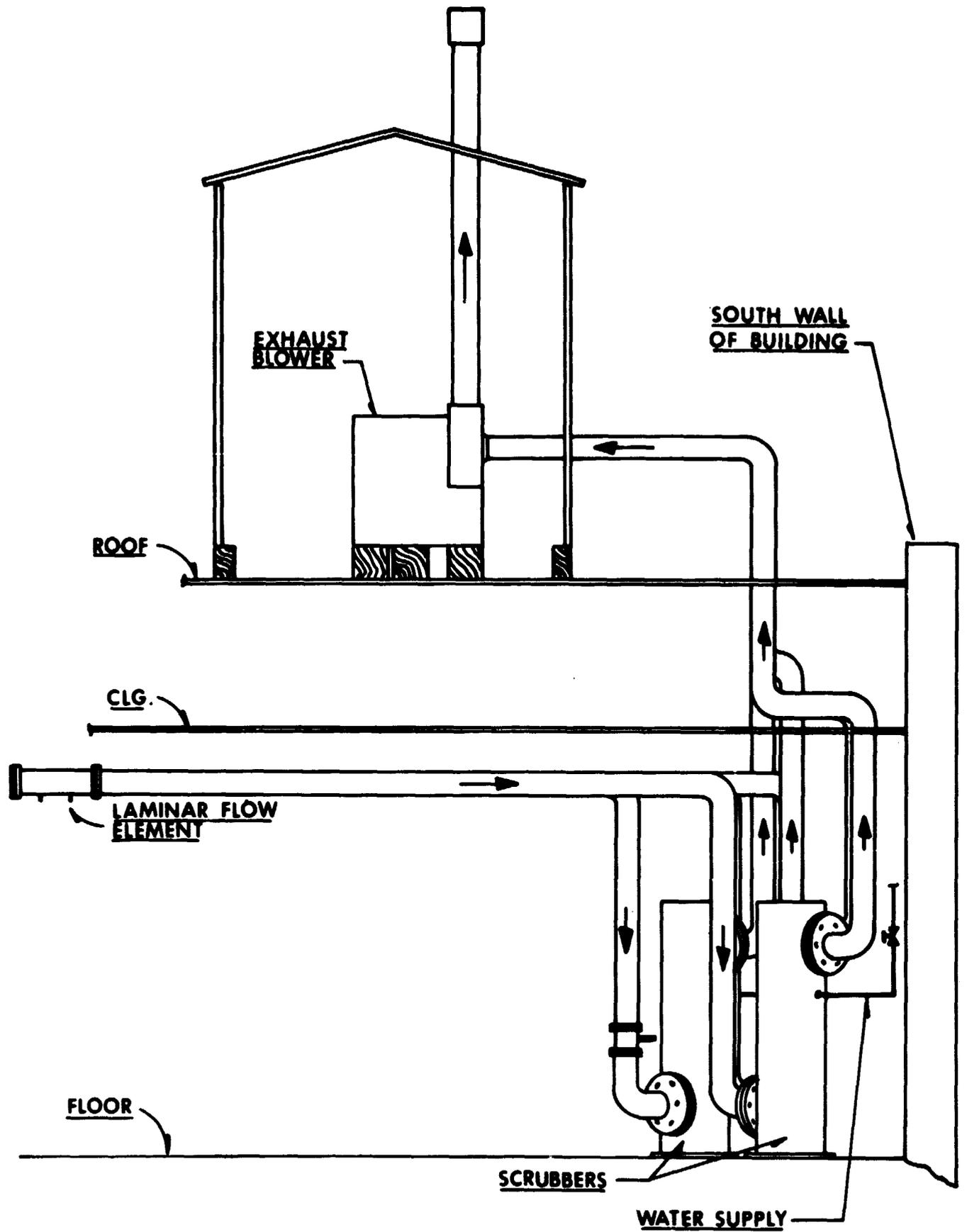
AMBIENT EXPOSURE CHAMBER
VENTILATION SYSTEM
ELEVATION VIEW

Figure 5

c.   Improved Flow Measurement and Control

Laminar flow elements were installed in each exhaust line for accurately measuring the chamber air flow over a range of 0 to 100 scfm. These flow elements replaced an orifice plate meter with a limited flow measuring range of 10 to 40 scfm. Differential pressure transmitters are used to transmit the flow proportional signals to the ambient laboratory control panel (CP-1) where the flow rates are recorded for each chamber. In addition, each exhaust flow will be controlled from the control panel by means of a flow recorder-controller. To provide flow control, four pneumatic actuators, originally used to control duct heaters in the ambient laboratory, were modified to actuate butterfly valves. These valves are installed in each chamber exhaust line. Either automatic or manual control of chamber exhaust flow is available.

d.   An Effective Warning and Evacuation Alarm System

The emergency warning light system for the ambient chambers was completely redesigned to reflect changes in both the equipment in the ambient laboratory and changes in building design due to the construction project. The warning lights were extended to cover not only additional points in the ambient laboratory but also stations adjacent to the ambient laboratory. Activation switches were located at the north and south double doors of the ambient laboratory. These doors have been designated as the primary evacuation exits in case of emergency.

A wall and doorway were designed which would isolate the ambient laboratory from the area connecting the new altitude laboratory with the remainder of Building 79, because access to the ambient laboratory is restricted during experiments with hazardous materials. With this wall installed, travel from the office area or clinical laboratories to the altitude laboratory areas is unrestricted, even during experiments with hazardous compounds. Figure 6 shows the revised laboratory layout and warning light system.

e.   A High Volume Laboratory Emergency Exhaust System

In conjunction with the emergency warning light system, a high volume exhaust fan was installed on the roof of Building 79 above the ambient laboratory. This fan has an exhaust rate of 7500 cfm which provides a complete change of air in the ambient laboratory in approximately two minutes. Six air intakes are situated approximately seven feet above the floor in the ambient laboratory providing exhaust from around each chamber as shown in figure 7. The emergency blower is activated by the switch used for initiating the warning light evacuation signal. A 20-foot stack, located on the roof of Building 79, is of such design that no cap is required on the top of the duct. The design provided a vertical linear air speed of approximately 2000 fpm which has resulted in adequate elevation and dilution of exhausted contaminants to prevent their capture by building ventilation supply intake blowers.

CUTLER HAMMER MAGNETIC STARTER

5 KVA TRANSFORMER FUSE BOX

TO C B 18

CONTROL SWITCHES

CONTROL BOX

PP

CONTROL SWITCHES

A

C

B

D

EMERGENCY EXHAUST BLOWER ON ROOF

M
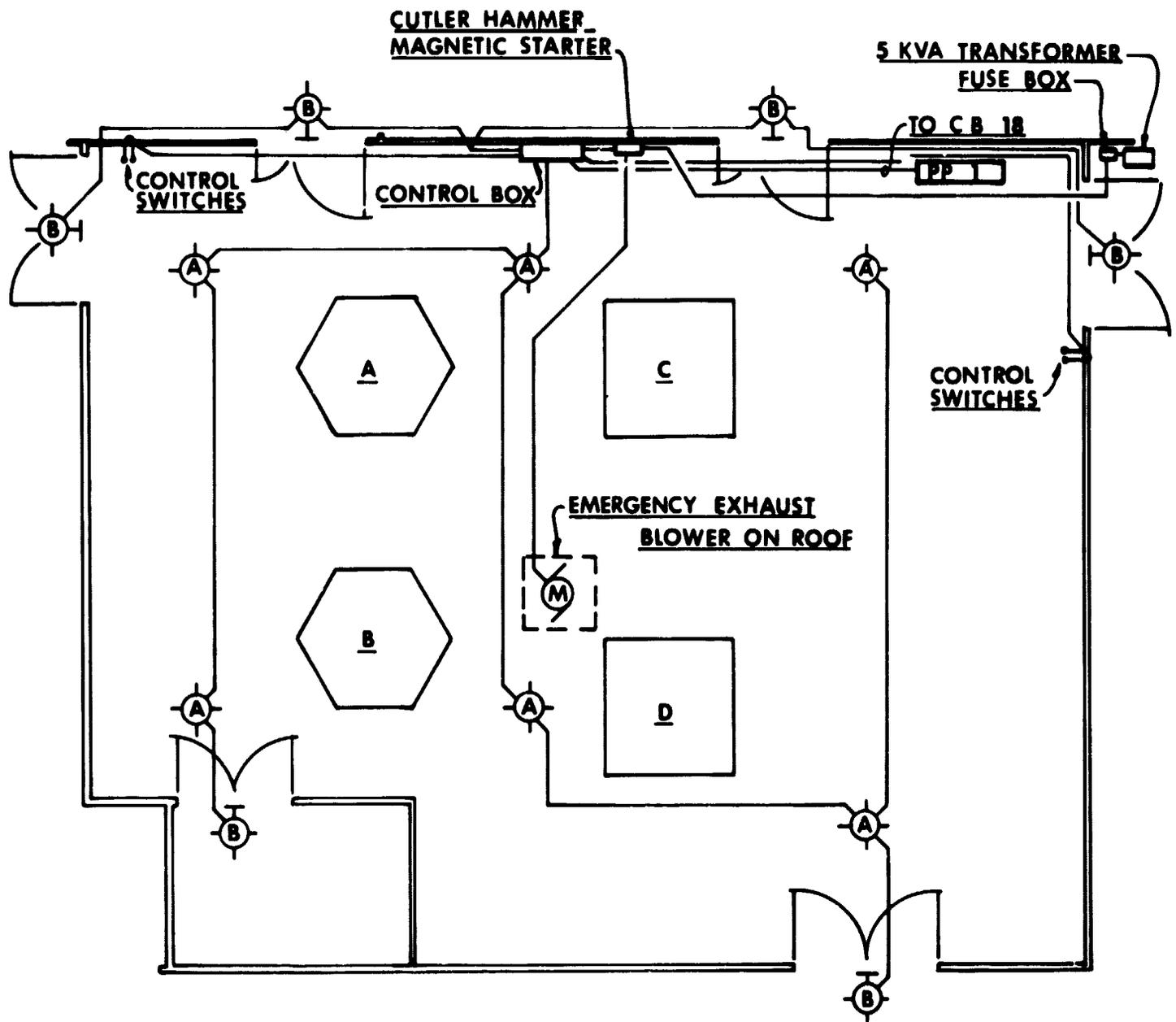
-Ⓐ- RED SPOT LAMP

Ⓑ- "HAZARD DO NOT ENTER"- LIGHT FIXTURE

EMERGENCY WARNING LIGHT SYSTEM

Figure 6

RM 153

UP TO
ROOF

12"X 18"(TYP)

A

B

C

D

GUY WIRES

20'-0"

ROOF

24"X 36"

24"X 24"

18"X 24"

CLG

18"⌀ (TYP)

AMBIENT LAB.

FLOOR
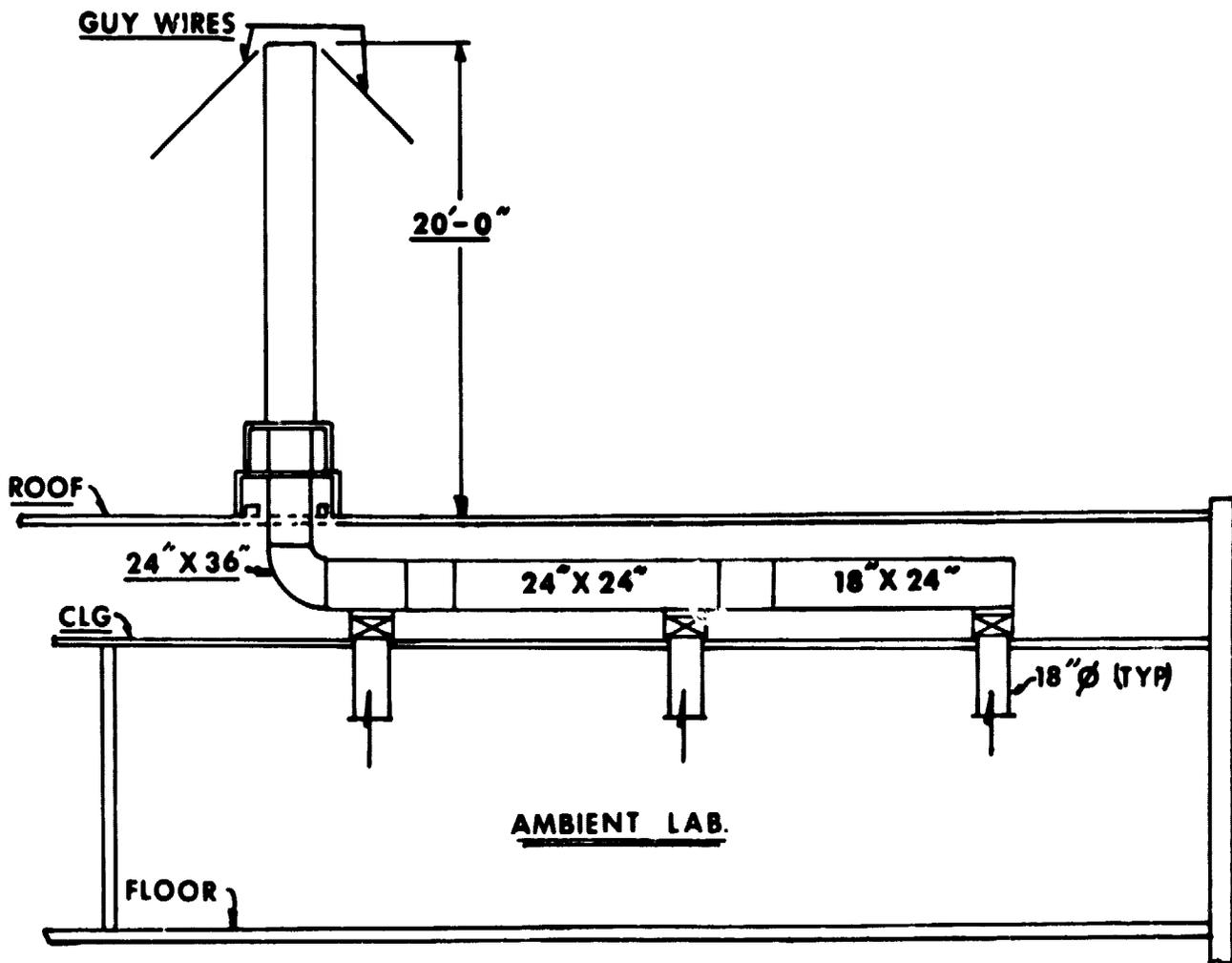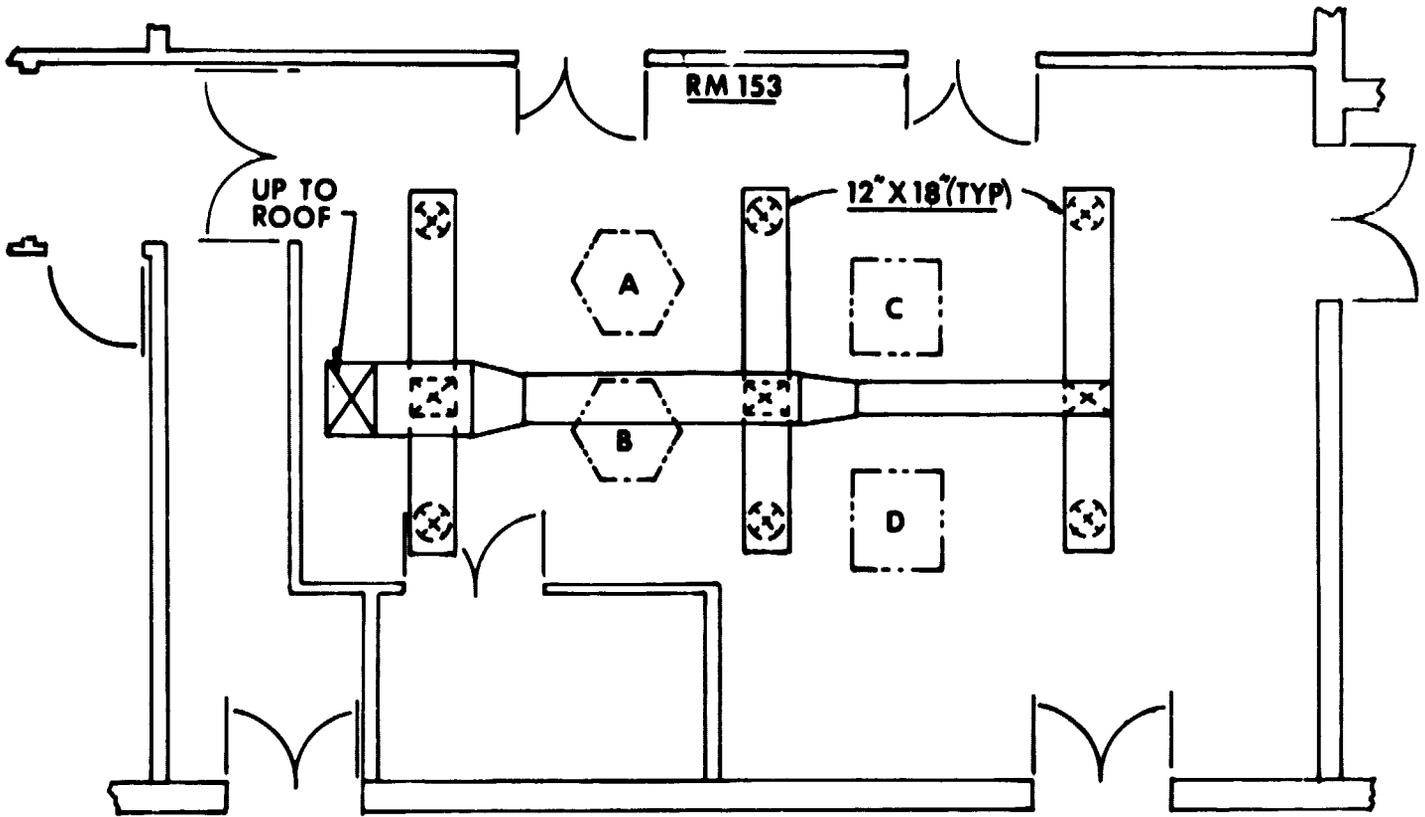
EMERGENCY EXHAUST SYSTEM
AMBIENT LABORATORY

Figure 7

24

f.   Improved Temperature and Humidity Control

Experience had shown that control over temperature and humidity of the air entering the ambient exposure chambers was marginal. Proper temperatures for chilling and heating of the intake air could not easily be attained since the intake air ducts were not insulated, and there was execessive heat exchange with the environment. All input air ducts were insulated from the air chiller to the chambers, providing a solution to the problem. At the same time, the laboratory air distribution ducts were also insulated to maintain the desired temperature of the conditioned air.

g.   Contaminant Generation Station Exhaust Ventilation

Four-inch stainless steel exhaust piping was installed to provide exhaust ventilation from the contaminant generating stations between the pairs of Rochester Chambers and Longley Chambers. The duct is exhausted by a roof-mounted blower, previously utilized for the ambient preconditioning chambers which were relocated to Building 429. The exhaust capability of the ventilation system for the contaminant generation stations is 300 cfm. In addition, a six-inch welded stainless steel exhaust system was designed for the exhaust hood used for acute toxicity exposures of rodents in bell-jar chambers. This installation required the use of a separate blower on the roof. Individual switches were provided for hood blower, roof blower and hood lighting.

Building 79A Modifications

The oxidizer dilution facility in Building 79A was extensively modified to permit safe and efficient dilution of oxidizers to be used in experiments conducted in the Thomas Domes. Particular attention was given to aspects of the operation which would affect the safety and health of operating personnel.

A blast shield has been designed and is being constructed to enclose hazardous gas supply cylinders for the dilution system. A remote control gas cylinder valve operator located behind the blast shield permits opening and closing of gas cylinders in the relative safety of the area outside the walk-in hood.

All components of the dilution apparatus are installed behind a concrete block blast wall. A large exhaust canopy covers the entire area of the walk-in hood and is connected to a blower on the roof of Building 79A. This was an existing blower modified to provide ample exhaust capability for the dilution apparatus area. Explosion-proof switches are mounted on the hood wall for controlling the blower.

Additional safety devices and equipment have also been installed. When conducting hazardous dilution, operating personnel are attired in protective clothing. A shower has been installed to permit personnel to insure the removal of contaminants incurred by accidental contact.

A system of manifolded compressed air cylinders was installed to provide air for breathing masks during dilution operations or in case of accidental spillages. The compressed breathing air is delivered to readily accessible locations in Building 79A.

An emergency eye wash was also available in the immediate area, and a warning system to inform personnel of potentially hazardous conditions in the area of Building 79A was installed. The warning system is activated by switches installed adjacent to the walk-in hood. Hazardous conditions are indicated by a flashing red light installed adjacent to the entrance of Building 79A and by signal lights installed approximately 300 feet from the building and near Building 79.

Standard operating procedures for the safe operation of the facility were formulated and submitted to several organizations for approval. These organizations were the Aeronautical Systems Division Safety Office (ASY); Wright-Patterson Air Force Base Fire Department (EWEF); Toxic Hazards Division, AMRL (MRT); AMRL Safety Officer; and THRU, SysteMed Corporation. Base Bioenvironmental Engineering (HWOB) also approved the procedures. Wright-Patterson Air Force Base firemen were made familiar with the location and functional operation of the dilution facility.

Building 95B Modifications

Building 95B has been converted into an instrument calibration laboratory. A section of the building is used for storage of critical spare parts for the electronic and pneumatic instrumentation while the remainder is used as a workshop.

With the acquisition of the Procedyne calibrator and installation of a pneumatic test panel, all recorders are being tested and calibrated in this building. A small instrument air compressor was purchased and installed to provide the necessary air supply to operate the equipment.

As a result of the modified use of the building there was an increase in electrical power requirements; therefore, a new 220 VAC - 50 amp electrical service was installed which provides sufficient power for all current equipment and for anticipated future requirements.

Work benches were installed with necessary accessories such as a bench vise, drill press, and a complete set of tools.

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With the above modifications and acquisitions Building 95B has been organized for the efficient performance by personnel in maintaining and operating the animal exposure facility.

Lithium Hydroxide Canister

The closed-loop life support system used in evaluating the toxicological effects of space cabin materials utilizes lithium hydroxide (LiOH) canisters for the removal of CO_2 from the animal exposure environment. Two canisters are used on each system and must be replaced periodically. The method of filling the canisters previously used resulted in excessive LiOH dust in the surrounding laboratory area. A self-contained dispenser, shown in figure 8, was designed to minimize the open handling of LiOH.

The dispenser was provided with a funnel shaped hopper designed to accept a standard 125 pound barrel of LiOH. The hopper and barrel are inverted in a completely enclosed housing which is approximately 5 feet long by 5 feet high. The bottom of the hopper fits into a slide valve which is operated from outside of the enclosure. During filling operations an empty canister is placed in the enclosure immediately below the slide valve, the enclosure door is sealed and the slide valve is opened. An observation window in the enclosure permits the operator to view the filling process. When the canister is filled to the proper level, the sliding valve is closed stopping the flow of LiOH. The enclosure is equipped with an exhaust blower which removes dust particles and fumes from the unit. Heavier particles fall to the bottom of the enclosure into a removable drawer which may be emptied at a later time. The unit was designed with a waste hopper compartment unit to permit the collection of the used LiOH and the storage of both used and unused canisters.

Thomas Dome Communications System

A communications system was designed and installed in the newly constructed Thomas Dome facility. Several design changes from the existing Thomas Domes were incorporated into the system. Particular advantages provided by the redesigned system are simplified operating controls, utilization of standard Air Force Communications components in the basic system, independent communications available in each dome, automatic voice-operated tape recording of all activities utilizing the system, capability of switching the interconnecting airlock into operation with any dome and an emergency power supply.

The system consists basically of a station for the control panel operator, four identical dome stations, an interconnecting airlock station and a station in the prebreathing room. An additional station is planned for the control panel operator to provide independent communications for two simultaneous dome flights.

TO EXHAUST BLOWER

BARREL HOPPER

FILL VALVE HANDLE

USED LITHIUM
DUMP PORT

OBSERVATION WINDOW

CANISTER FILLING
COMPARTMENT

LITHIUM
WASTE DRAWER

COMPARTMENT FOR
FILLED CANISTERS

COMPARTMENT FOR
USED LITHIUM
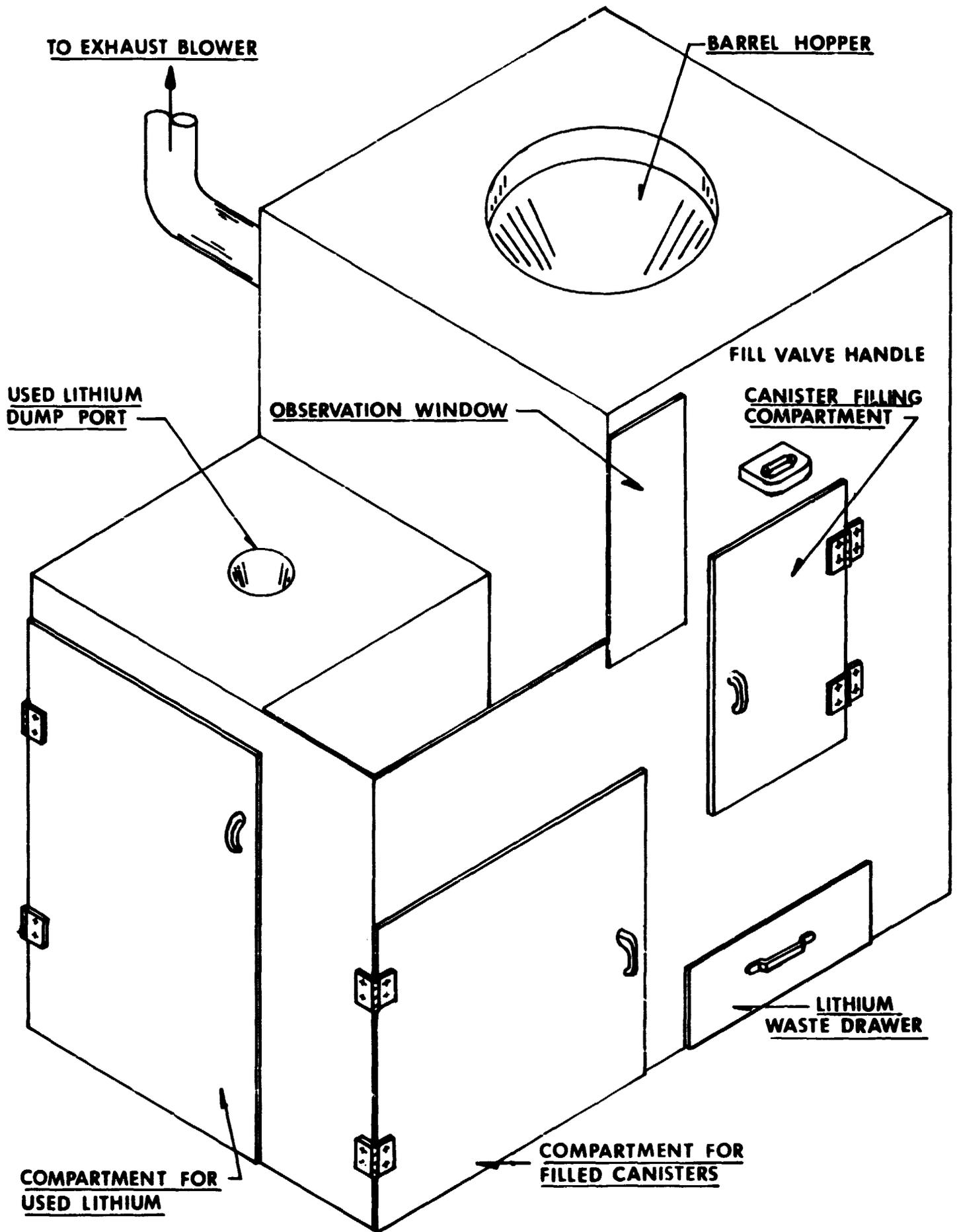
LITHIUM HYDROXIDE HOOD

Figure 8

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The station installed at the main control panel contains the emergency power supply, tape recorder and power controls. Plug-in connections are available for two headsets at this location. Switching facilities are included which enable the control panel operator to independently connect his station with an individual dome or the interconnecting airlock. The tape recorder used in this station is completely automatic and requires no action of the operator. The recorder is voice-actuated and has an automatic-reversing attachment. The tape reel used allows 6 hours recording time which is sufficient for the longest dome flight now anticipated. A window was provided on the enclosure for the dome operator to determine that the recorder is operational.

Each of the four individual dome communication stations is identical. These stations, installed in the basement adjacent to the airlock of each dome, are completely enclosed and have no operating controls. There are four substations, containing plug-in connections for three headsets connected to each dome station, located as follows:

1.   Dome Interior; First Floor
2.   Observer B Station; First Floor
3.   Dome Airlock Interior; Basement
4.   Observer B Station; Basement

Two additional substations will be installed on the periphery of the dome on the first floor. Each of the systems may be used independently.

A separate station was installed for the central interconnecting airlock. This station is located adjacent to the Observer B station and porthole of the central airlock. It is provided with two headsets and a switch to connect with any one of the four domes. Substations, with provisions for connecting three headsets each, are installed at the main airlock entrance and also the interior of the airlock. Additional substations will be installed at each of the remaining airlock windows.

The new oxygen prebreathing room has a communication station with 6 connecting headsets. These are divided into two groups of three. Each group may be independently switched to any of the domes or interconnecting airlock. Communications from the prebreathing station to the existing domes will be connected in the future.

Design features of the system provide extremely versatile and flexible communications capability to all areas of the new dome system and when interconnected with the existing facility, will provide complete audio capability for all activities involving the Thomas Domes. Figure 9 shows the physical layout of components on the first floor, and figure 10 shows all components in the basement areas.
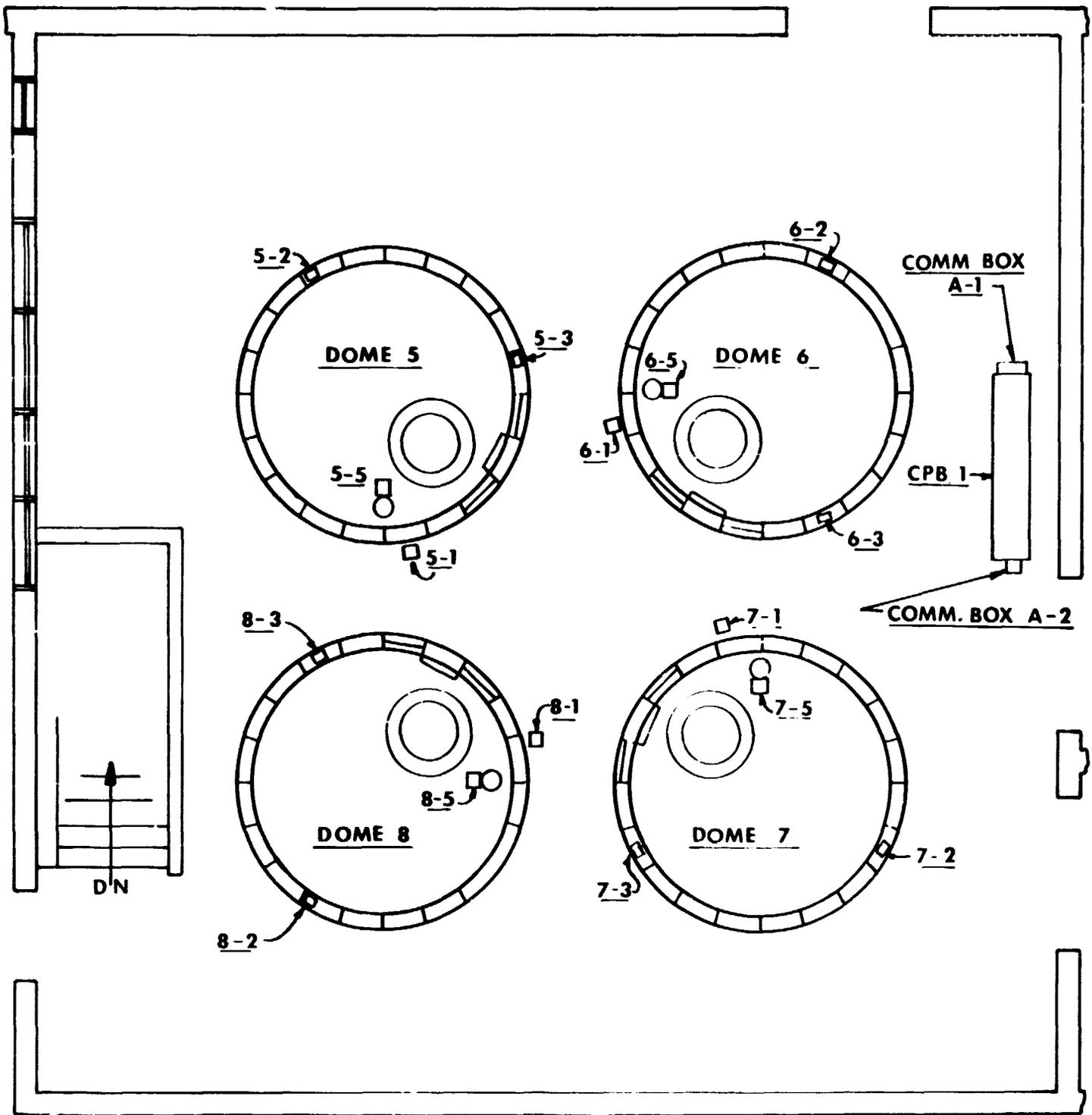
COMMUNICATION SYSTEM
MAIN FLOOR

Figure 9

COMM. BOX-10

JUNCTION BOX FOR FUTURE

U.P

6-4

6-6

COMM BOX-5

9-3

5-4

A.L.-5

A.L.-6

5-6

COMM.BOX-6

CENTRAL
AIRLOCK

COMM.BOX-9, 9-1

9-2

COMM.
BOX-8

9-5

DN

7-6

A.L.-8

A.L.-7

U.P

8-6

9-4

7-4

8-4

COMM.BOX-7
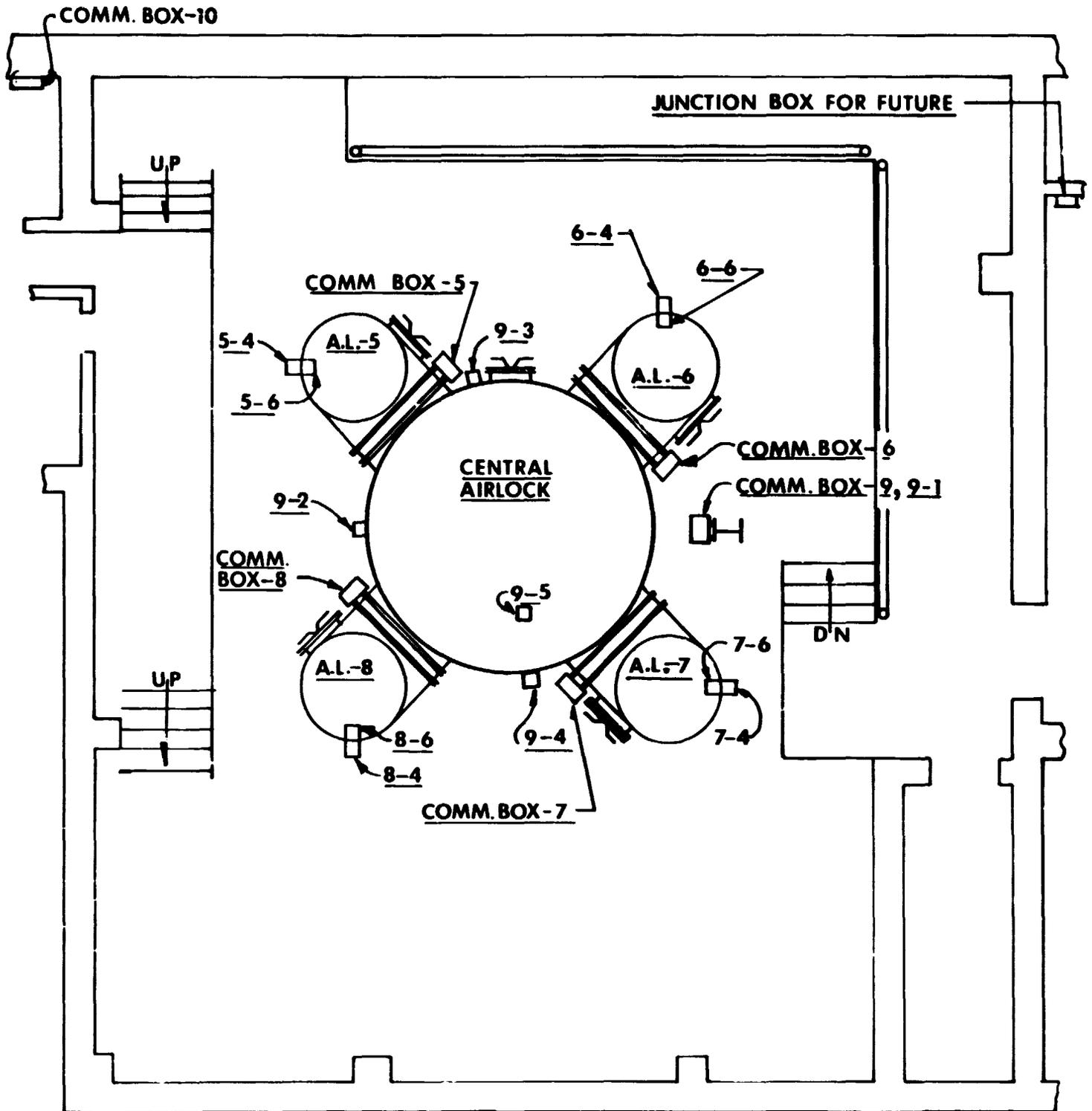
COMMUNICATION SYSTEM
BASEMENT

Figure 10

SECTION III

RESEARCH PROGRAM

The inhalation toxicology research program of the THRU covers a broad area of interest ranging from standard industrial hygiene toxicology problems to the more exotic but real problems of determining safe limits for continuous low level contaminant exposures in spacecraft atmospheres. The primary mission of the THRU program is to provide answers to these practical problems concerning the health not only of Air Force personnel but of the civilian population working with the same or related materials.

As in previous report periods, some of the research experiments discussed herein were initiated in the preceding year and some that were started this year will carry over into the next reporting period. Toxicity screening of space cabin construction material is a continuing project with individual experiments conducted whenever sufficient materials are made available for the testing.

Monomethylhydrazine

The investigation of monomethylhydrazine (MMH) acute toxicity was completed during the past year. Information concerning the effects of single exposures to high atmospheric concentrations of MMH and LC_{50} data from several species of animals has been reported elsewhere (reference 5) and will not be repeated here other than to define the principal effects.

High atmospheric concentrations of MMH have produced central nervous system effects in dogs, monkeys, rats and mice leading to convulsions which frequently result in death. There were no fatalities among animals exposed to any time-concentration (CT) dose of MMH that did not cause convulsive seizures. Another consistent effect of MMH observed at high dose levels was a transient hemolytic anemia characterized by a significant decrease in hematocrit, red blood cells and hemoglobin which persisted for several days postexposure.

On the basis of the LC_{50} data obtained in the acute toxicity experiments, an investigation of the validity of current emergency exposure limits (EEL) was undertaken. The purpose of this investigation was to determine whether the current untested EEL values were more stringent than necessary for personnel safety. A health or safety limit that is unnecessarily stringent results in excessive and wasteful costs for environmental control.

In this context, then, the MMH concentrations selected for FEL testing on the four selected species (rat, mouse, beagle dog, and Rhesus monkey) were based on a CT of 900 ppm-minutes. This CT value was approximately 25% of maximum nonlethal concentrations for the most susceptible species, the squirrel monkey, and was also five times higher than the current EEL values adopted by the NAS-NRC Advisory Center on Toxicology. The selected concentrations were 15, 30 and 60 ppm MMH for single exposure periods of 60, 30 and 15 minutes respectively.

Groups of 18 Sprague-Dawley rats (140-175 grams) and 18 ICR (Swiss origin) mice (25-30 grams) were exposed to the 900 ppm-minute doses of MMH vapor for 15, 30 and 60 minute periods. Groups of 9 unexposed control rodents were used for comparison with each test group. Additional groups equal in size and number were subjected to higher time-concentration exposures; namely, 150, 75 and 40 ppm MMH for 15, 30 and 60 minutes respectively.

The rats were weighed before exposure and at 1, 3 and 7 days postexposure. One third of the exposed and control rodents were killed at 1, 3 and 7 days respectively for evaluation of possible injury and reversibility. During the postexposure period all animals were fed ad libitum.

Twenty-four purebred female beagle dogs (6 to 9 months old) were also exposed in groups of 8 to each of the 900 ppm-minute test combinations. Postexposure observations were made for a period of 30 days on 2 MMH exposed and 2 control dogs for each CT group while the remaining dogs were killed at 1, 3 and 7 days for pathology evaluation. The groups of dogs held throughout the 30-day postexposure observation period were examined clinically and routine blood tests were made twice weekly. Necropsies were performed at the end of the observation period and tissue specimens were submitted for histopathology.

Comparable numbers of female monkeys (24) (Macaca mulatta) weighing 3 to 5 kilograms were exposed to the same time-concentration of MMH as were the dogs. Again most monkeys were killed at 1, 3 and 7 days postexposure for pathologic evaluation, and groups of 2 monkeys exposed to each MMH dose level tested were observed clinically for 30 days after which necropsies were performed and histopathology evaluations conducted.

Because of the reactive nature of MMH and the extremely small range of concentrations between the no-effect and effect levels seen in the preliminary experiment, a method of continuous analysis was required. The continuous monitoring of chamber MMH concentrations was accomplished by use of an electron capture instrument which measured the concentration of an aerosol formed by the reaction of MMH with trifluoracetic acid vapor.

This instrument is a self-contained monitoring system suitable for continuous analysis, in the parts per billion range, of acidic or basic vapors which can be reacted to form aerosols within the apparatus. An electrical signal generated by the electron capture detector was transmitted to a millivolt recorder. Typically, the instrument recorder response-time was only 12 seconds, permitting almost instantaneous readout of chamber concentrations.

No significant differences between MMH exposed and control rodents were observed at any of the six selected EEL test concentrations. The mean pre- and postexposure weights of the MMH exposed rats and their controls are summarized in table III. The three individual groups of the 2250 ppm-minute and 900 ppm-minute CT exposures have been lumped together since there were no significant differences between them. Organ to body weight ratios are presented in table IV for the same groups of animals. Again, no significant differences were observed at either the 900 ppm-minute or the 2250 ppm-minute levels.

No effects on body weight were observed in either dogs or monkeys exposed to the three 900-ppm-minutes MMH exposure systems. At necropsy both species exhibited mild transitory changes which consisted of minimal congestion with slightly increased pigmentation of the renal cortex. These changes had completely resolved by the 30-day sacrifice period.

Histopathologic evaluation of tissues of both dogs and monkeys necropsied at 1, 3, 7 and 28 days postexposure showed no significant differences from control animals. An additional group of dogs exposed to 15 ppm MMH for one hour for evaluation of clinical chemistry parameters was also negative with respect to pathologic effects.

No clinical signs or symptoms of CNS changes could be observed in any of the four animal species exposed to MMH in the EEL test series. There was also no indication of respiratory irritation as had been observed during MMH inhalation exposures near the LC_{50} concentration. Biochemical determinations on blood specimens taken from both dogs and monkeys exposed to the three concentration time periods remained within normal ranges.

An important factor in considering the establishment of inhalation exposure limits for MMH is its rapid oxidation in air and in the animal. Air oxidation begins immediately and is relatively complete within one hour as reported by Vernot et al (reference 15). The primary oxidation products of MMH in air are methane and nitrogen. Small amounts of other carbon containing compounds are also formed which include CO_2. Dost et al (reference 3) have described the metabolic fate of MMH in rats using ^{14}C labeled material injectioned intraperitoneally. The in vivo oxidation of MMH reaches a maximum rate within two hours and is essentially complete 3-4 hours postinjection.

TABLE III

Mean Body Weights of Albino Rats
Exposed to Monomethylhydrazine
(weight in grams)

| | Days Postexposure | | | | No. of |
	0	1	3	7	Rats
Exposed: CT = 2250	153	155	166	196	54
Controls	148	155	169	197	27
Exposed: CT = 900	163	167	180	203	54
Controls	163	170	182	205	27

TABLE IV

Mean Organ to Body Weight Ratios of Albino Rats
Exposed to Monomethylhydrazine
(organ weight/100 gram body weight)

	Heart	Lungs	Liver	Spleen	Kidney	No. of Rats
Exposed: CT = 2250	0.408	0.695	4.391	0.438	0.950	54
Controls	0.397	0.662	4.537	0.428	0.940	27
Exposed: CT = 900	0.426	0.667	4.638	0.416	0.938	54
Controls	0.406	0.656	4.583	0.408	0.924	27

Approximately 30% of the metabolized MMH is excreted as methane and about 10% as CO_2. The bulk of the remaining carbon containing metabolite appears in the urine.

These findings on the metabolic fate of MMH with respect to rate of decomposition agree with the reported findings of Reynolds and Back (reference 11). The performance decrement induced in primates by MMH began 1-2 hours after injection and decreased significantly 4-5 hours after injection.

The induction of performance decrement in primates required an MMH dose of 2.5 mg/kg. Calculation of the maximum possible inhaled dose of MMH in the studies reported herein shown it to be 0.5 mg/kg for the primate or 20% of the dose required for performance decrement.

In view of the negative findings in all species from MMH inhalation exposures of 900 ppm-minutes and in both rats and mice at 2250 ppm-minutes and the safety factor for performance decrement, we recommended an upward revision of the Emergency Exposure Limit values for monomethylhydrazine as shown below:

Minutes	PPM MMH
10	90
30	30
60	15

These recommendations were presented to the Committee on Toxicology of the Toxicology Advisory Center of the National Academy of Sciences - National Research Council in January 1969 for their consideration.

Ethylene Glycol

A provisional 60-minute emergency exposure limit for ethylene glycol has been established by the National Academy of Sciences - National Research Council Committee on Toxicology. This limit of 100 millimole/25M³, equivalent to 258 mg/M³ ethylene glycol, is published in the Space Science Board of NAS-NRC report of June 1968 entitled "Atmospheric Contaminants in Spacecraft." In the documentation for establishment of the provisional limit the panel referred to unpublished data which indicated the possibility of serious eye injury from repeated exposure to ethylene glycol vapor. The panel recommended that high priority be given to further research in this area. The Toxic Hazards Research Unit was requested to conduct such research.

Four rabbits, 30 rats and 20 guinea pigs were continuously exposed to a 5 ppm ethylene glycol atmosphere within a Rochester Chamber. An equal number of control animals of each species were housed in another Rochester Chamber being subjected to the same environmental conditions as the test animals with the exception of ethylene glycol vapors. The initial group of rabbits developed a severe enteritis resulting in death for a significant portion of both test and control animals. Consequently, a second group of rabbits (4 test and 4 control) were added to the experiment on the 40th day of continuous exposure. This second group of rabbits, called Group B, were exposed to the 5 ppm ethylene glycol atmosphere for 17 days; all original animals had a total of 57 days continuous exposure.

The concentration of ethylene glycol in the exposure chamber was continuously monitored by means of an autoanalyzer technique using a modified Schiff's reaction with acid fuchsin. The mean concentration of ethylene glycol for the 57-day exposure was 5.4 ppm (S. D. $+0.99$) with a range of 4.1 - 7.8 ppm. Exposure was continuous but the chamber concentration was unstable for a 30-minute to 1-hour period each day when the door was opened for routine cleaning and feeding of the animals. During this period animals were weighed and their eyes examined in accordance with the experimental protocol.

All experimental animals, both exposed and control, were given opthalmologic examinations for corneal defects prior to the exposure period. The examinations were made with the slit-lamp and the fluorescein - UV lamp methods. No corneal opacity was noted although some rabbits exhibited simple scratches which could be seen by the fluorescein method. Subsequent examinations of the animal's eyes were conducted at weekly intervals. Upon a suggestion from Dr. Elliott Harris of NASA, Group B rabbits were examined daily for the first week of exposure.

No differences were seen in the corneal surface of the eyes of rabbits, rats or guinea pigs exposed to 5 ppm ethylene glycol continuously for 57 days. Group B rabbits, examined daily, exhibited minimal cloudiness of the surface layer of corneal epithelium. This change occurred during the first three days of exposure and disappeared with further exposure. At the conclusion of 17 days continuous exposure to the 5 ppm ethylene glycol atmosphere there were no differences between the Group B control and exposed rabbits.

The ethylene glycol exposure had no effect on animal growth. The Group A rabbits that were not affected by the enteritis showed normal growth patterns as did both guinea pigs and rats. The growth rate of the rats is shown in figure 11.

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GROWTH RATE OF MALE RATS CONTINUOUSLY
EXPOSED TO 5 PPM ETHYLENE GLYCOL
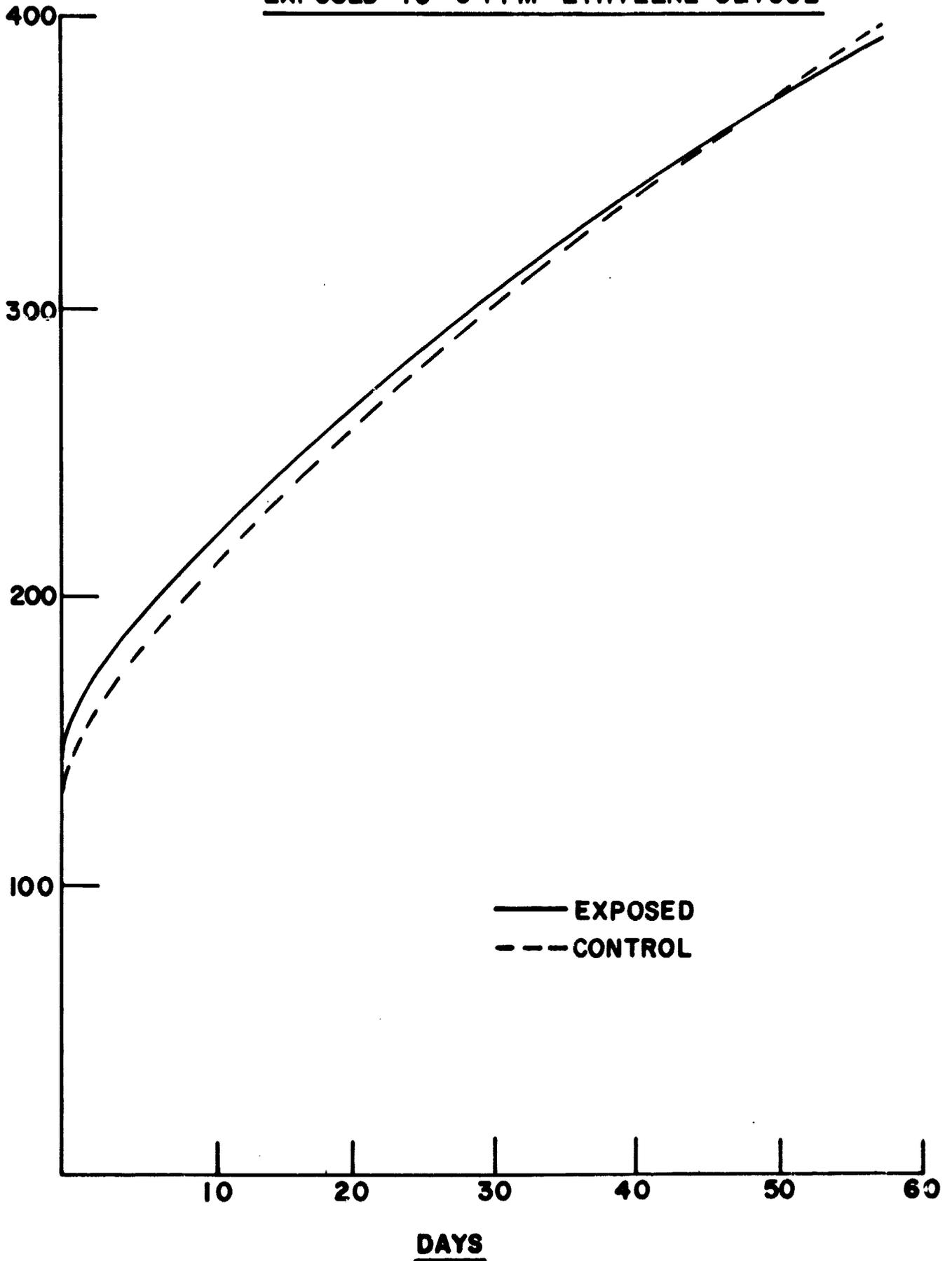
Figure 11

All groups of original test subjects along with their controls were necropsied at the end of 57 days exposure. The surviving Group A rabbits, one test and two control, as well as every fifth rat and guinea pigs were submitted for post-mortem examination. Two Group B test rabbits and 2 controls were also necropsied at the conclusion of the experiment.

The remaining Group B rabbits were used for additional testing. Concentrated ethylene glycol was instilled in the right eyes of two rabbits; the left eye served as a control. A 10% solution of ethylene glycol in water was instilled in the right eyes of the remaining rabbits. Again the left eye served as a control. The eyes of the rabbits were observed for 7 days and then the animals were necropsied and the ethylene glycol exposed corneas subjected to histopathologic examination.

No significant corneal damage, related to either the 5 ppm vapor exposure or the topical application of ethylene glycol, could be identified. Although minimal inflammatory changes were occasionally found at the corneal border, these changes occurred in control animals as frequently as in the corneas of exposed animals. No instance of corneal ulceration was seen in any of the experimental animals, either exposed or control.

The experimental results obtained by the Toxic Hazards Research Unit do not substantiate the possibility of eye injury from exposure to ethylene glycol.

Nitrogen Trifluoride

Animal studies investigating the acute toxicity of NF_3 were initiated during the 1967-1968 report period and preliminary information concerning mortality, methemoglobinemia and blood turbidity development was presented in the last THRU annual report. Experiments leading to the determination of LC_{50} values and emergency exposure limits were continued and concluded during the past year.

LC_{50} Experiments

Rats, mice, Rhesus monkeys and beagle dogs were exposed to various concentrations of NF_3 for 15-, 30- and 60-minute periods. The LC_{50}'s calculated from the mortality data so obtained using the "moving average" technique (reference 14) are listed in table V.

TABLE V

Nitrogen Trifluoride LC$_{50}$ Values for Various Species

Species	LC$_{50}$, ppm		
	60 Minutes	30 Minutes	15 Minutes
Mice	7500	12300	19300
Rats	6700	11700	26700
Dogs	9600	14000	24000
Monkeys (6-8 lbs.)	7500	14000	24000

The effect of NF$_3$ on all species appeared to be quite comparable except in the case of the 15-minute mouse exposure. The mouse exposure data indicated the possibility of a different CT relationship, specifically from that of rats, and of an inversion of relative toxicity at longer times. This appeared to be confirmed by exposures of rats and mice to 4500 ppm NF$_3$ for 120 minutes with resultant mortalities of 2/10 for mice and 9/10 for rats.

Individual survival of dogs and monkeys seemed to be dependent on the amount of methemoglobin formed during exposure or, conversely, on the concentration of oxyhemoglobin remaining, as measured on blood sampled immediately after exposure. In general, surviving animals demonstrated oxyhemoglobin values in excess of 4 g %, while animals with greater than 75% conversion of oxyhemoglobin to methemoglobin invariably died. This substantiates the conclusion that mortality resulting from acute exposure to NF$_3$ is due to anoxia.

Monkeys and dogs surviving acute NF$_3$ exposures showed similar patterns of hematological change: rapid and extensive formation (immediately postexposure) and the subsequent disappearance (10 hours) of methemoglobin; parallel increase of turbidity and Heinz bodies to a maximum on the 2nd day, leveling off to the 9th day, then decreasing to zero by the 20th day; parallel slow decreases in RBC, HGB and HCT to a minimum somewhere between the 9th and 17th day followed by slow recovery to normal values by the 40th day. The minimum values observed in RBC, HGB and HCT were accompanied by a concurrent increase in reticulocyte counts.

The close correspondence of Heinz body counts and turbidity in the hemolyzed samples led to the speculation that they were, in fact, manifestations of the same phenomenon. In order to test this hypothesis, turbid hemolyzed blood from a dog exposed to an LC_{50} concentration of NF_3 was centrifuged, and the residue treated with cresyl blue, the Heinz body stain. This demonstrated that the residue consisted primarily of quasi-crystalline, stained granules indistinguishable from Heinz bodies in appearance.

Gross and histopathological findings in those animals that died during or after exposure were consistent with a diagnosis of anoxia and massive methemoglobin formation. These findings included lung reddening, congestion, edema, focal hemorrhage, and liver, kidney and spleen congestion. Pathology in surviving animals could be explained by the anemia following exposure, including renal tubular necrosis and regeneration and hemosiderin pigments in liver and renal tubules.

Emergency Exposure Limits

The present emergency exposure limit set by the NAS-NRC for NF_3 is 100 ppm for 30 minutes and 50 ppm for 60 minutes (3000 ppm-minutes). The evidence from the LC_{50} experiments indicated that this limit was unrealistically low and experiments were planned which might give results justifying raising of the limits. Because the primary acute effect of NF_3 is anoxia due to methemoglobin formation, there appeared to be sufficient similarity to the acute effects of carbon monoxide to use the EEL.'s cf this extensively investigated compound as the starting point to define NF_3 test concentrations. Since the CO EEL's are based on formation of 15% carboxyhemoglobin, a series of NF_3 exposures at 15, 30 and 60 minutes were conducted to determine the CT value necessary to produce 15% methemoglobin. These CT values for dogs and monkeys were close enough to 120,000 ppm-minutes to use that value as the prospective EEL dose.

Accordingly dogs and monkeys were exposed to nominal concentrations of 2000, 3500 and 7000 ppm NF_3 for 60, 30 and 15 minutes respectively. The animals were held for 28 days postexposure during which time the same hematological parameters obtained in the LC_{50} studies were measured. Although the monkeys showed no difference between control and exposed values for these parameters, all groups of exposed dogs suffered decreases of approximately 15% in HCT, HGB and RBC by the 10th day. Such a decrease was deemed sufficient to invalidate the proposal of 120,000 ppm-minutes as an EEL dose. Therefore, new range-finding studies with dogs were undertaken which indicated that a 30,000 ppm-minute dose did not cause significant changes in the three hematological parameters of interest. A little more than 2% methemoglobin was measured in the blood immediately after exposure.

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Dogs, monkeys, rats and mice were exposed to the selected EEL dose for 15, 30 and 60 minutes with measurements of HGB, HCT, RBC and body weight over the 28-day period selected previously. Organ weights were obtained and gross pathological examinations were made at the end of the postexposure holding period. Organ to body weight ratios have not yet been obtained but all other measurements showed no difference between controls and experimental animals both during the postexposure holding period and at termination of the experiment. If no adverse effects are seen in organ to body weight ratios and histopathology, the 30,000 ppm-minute dose level will be recommended as an upward revision for the current NF_3 EEL.

Mixed Gas-Reduced Pressure Environments

An 8-month investigation on the effects of continuous exposure to an environment consisting of a 68% O_2 - 32% N_2 gas mixture at 5 psia pressure was completed during the past year. This investigation was undertaken to repeat a previous study in which one species, the dog, exhibited inverted A/G ratios beginning during the third month and persisting throughout the remainder of the exposure. The purpose of the repeat experiment was to verify that the previously observed changes and trends were actually the result of exposure to the mixed gas environmental conditions. Consequently, all dogs selected both for the dome exposure and as controls had high normal A/G ratios which had been measured repetitively during the preexposure period.

During the third month of exposure occasional test dogs demonstrated inverted A/G ratios. These inversions of ratios were not repeated and after 8 months of continuous exposure there were no apparent differences between the control and the exposed dogs. Exposed male rats, however, exhibited a depressed growth rate as seen in figure 12 which was significant at the 1% level. This trend, as shown in figure 13, was also seen in the previous experiment although it was not statistically significant.

The conclusion that may be drawn from the 2 experimental exposures to a mixed gas-5 psia environment is that no significant effects were seen in dogs, monkeys and mice. The only real effect observed from exposure to these environmental exposure conditions was that of depressed growth in rats. The depression of growth was not, however, associated with any other pathological change.

Carbon Tetrachloride

A short experiment on carbon tetrachloride (CCl_4) toxicity was conducted to clarify a point of confusion caused by an earlier series of experiments in which the toxicity of this chlorinated hydrocarbon was compared at reduced and ambient pressure conditions.
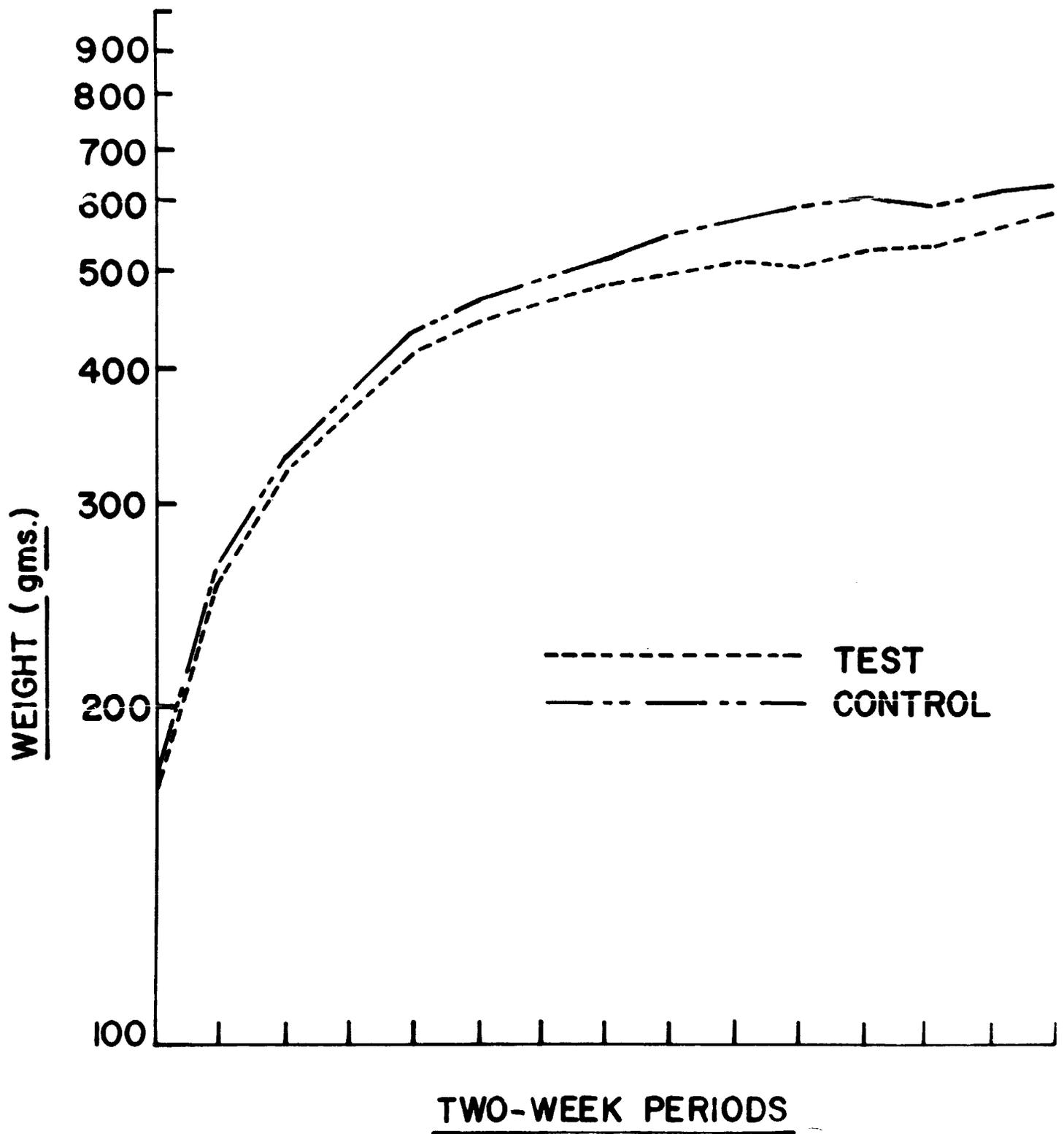
Figure 12. Male Rat Growth; Repeated 8-Month Mixed-Gas Study

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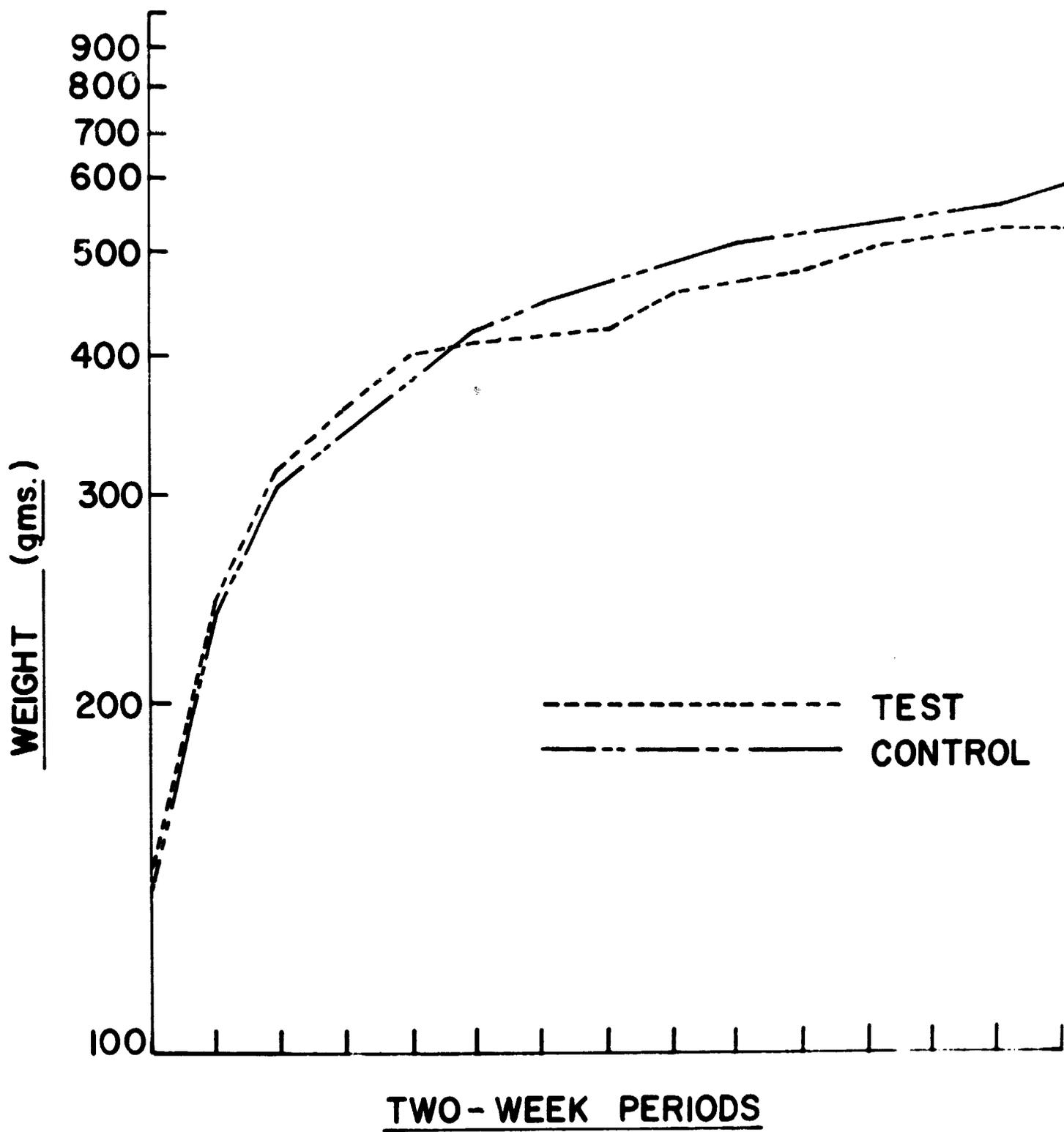
Figure 13.   Male Rat Growth; Original 8-Month Mixed-Gas Study

A group of 40 mice was continuously exposed to CCl_4 concentrations of 590 mg/M^3 in a 5 psia-100% O_2 environment for two weeks. This experiment was conducted to complete a series begun in 1964 and was a repeat of a similar experiment in which 39 to 40 exposed mice died within the exposure period. The delay in conducting this repeat experiment was due to priority assignments as well as the long period of experimental shutdown while the Thomas Domes were modified to meet current fire safety regulations.

The results of the experiment verified the initial assumption that the original observed mouse mortality was not due to the exposure to CCl_4 but was associated with an unidentified type of acute pulmonary infection. The mortality experience in the current experiment was 1 of 40 and the primary gross pathologic finding was fatty infiltration in liver cells. The fat deposits were identified by the oil red "O" staining technique.

Oxygen Difluoride

In February 1969 the exposure of a young man to oxygen difluoride (OF_2) was brought to the attention of the THRU staff through an emergency call for information concerning the toxicity of this compound and for therapeutic measures to be taken.

A chemistry student in a large northern university working with OF_2 was exposed when he had an accident. A small explosion in the gas transfer system due to a reaction between OF_2 and benzene used to clean the tubing resulted in knocking over the OF_2 cylinder. The cylinder valve was damaged and could not be closed. The student, unaware of the health hazard of OF_2 gas, picked up a wrench, leaned over the cylinder leaking OF_2 and closed the main valve. He left the laboratory and got another student to take him to the health clinic when he began experiencing some irritation. He was consequently taken to a nearby hospital and placed under intensive care.

Members of the THRU investigated this accidental exposure and concluded that his exposure had been approximately two minutes to a concentration in the neighborhood of 1000 ppm. Since this was expected to be a lethal dose (reference 6) we were surprised to see the patient survive, but he did. He suffered some shortness of breath and soreness of the chest. At the time of greatest discomfort, he was relieved by two or three deep breaths of oxygen from a hand mask. On rechecking the calculations of the possible exposure, we again concluded that the student had been exposed to a concentration of approximately 1000 ppm.

Since investigations of OF_2 toxicity were scheduled to be conducted in the THRU laboratory in the near future, some preliminary experiments were undertaken immediately. The unexpected survival of the man exposed to an

estimated OF_2 concentration of 1000 ppm might indicate a significant difference of human response to this compound from various rodent species which had been tested previously. There were no indications in the published literature of other human exposures or of experimental exposures to species other than the rodent.

Small groups of rats and single monkeys were exposed to nominal concentrations of OF_2 in the range of 5 to 20 ppm. Single 10-minute exposures of rats to a nominal 5 ppm OF_2 resulted in 100% mortality within 5 minutes post-exposure. A monkey exposed to 10 minutes to 5 ppm OF_2 survived 15 minutes longer than one exposed to 20 ppm OF_2 for the same length of time. The monkey exposed to the nominal 5 ppm concentration died 61 minutes postexposure. An automatic analytical method for the determination of low concentrations of OF_2 is being developed for further investigation of this material which appears to be highly toxic.

Wistar Rat Study

The Sprague-Dawley rats used in our research program during the past years were showing an increasing frequency of chronic murine pneumonia which interfered with the histologic evaluation of pulmonary effects of toxicants being tested. A source of Wistar rats free of pulmonary pathogens had been used in the THRU until the program of reduced pressure experiments in the Thomas Domes was started. In the initial dome experiments conducted at 5 psia-100% oxygen conditions the Wistar strain rats had exhibited a spontaneous 15-20% mortality rate. No pathogenic cause for these deaths was ever identified but the mortality rate was reproducible in successive groups of rats. The press of scheduled experimental commitments prevented further study of this phenomenon at the time it was observed and other strains of rats were tested and found suitable for use in the reduced pressure-100% oxygen experiments.

The objective of a series of experiments using Wistar strain rats in a 5 psia, 100% O_2 environment was to determine whether these animals could be made to thrive under such conditions by holding them in ambient air for varying periods of time under the same food and water regimen that they would have in the domes. Since rats from this source were known to have negligible incidence of chronic murine pneumonia, they were preferable to the animals we had been using.

The results of these experiments showed that holding the Wistar strain rats for three weeks prior to placing them in the domes makes them as adaptable at altitude conditions as the Sprague-Dawley strain we were using.

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Toxicity Screening-Spacecraft Materials

A continuing program of toxicity screening of space cabin construction materials is conducted for both Air Force and NASA space flight systems. As materials are received for screening, they are assembled into groups of 15 to 20 and prepared, if necessary, according to manufacturer's directions. The preparations include painting shellacs, varnishes, and other surface coating materials on metal foil, and mixing and curing plastic polymer formulations.

The prepared group of materials is placed in a vacuum oven which is part of the closed-loop life support system previously described (reference 4). The oven is heated to 155 F at 5 psia and then the breathing atmosphere is passed through the oven and introduced into the animal exposure portion of the closed loop with the added gas-off products.

During the past year, 7 groups of Apollo Materials and 1 group of Cabin Materials were subjected to 7-day toxicity screening studies and found nontoxic under the environmental conditions tested. Two additional groups of Apollo Materials (V and W) are currently undergoing the 7-day screening procedures. A 60-day screening study was conducted for a group of 68 Apollo Materials. Again no detrimental toxic effects on this group of materials (T) were observed either during the 60-day exposure period or the 4-week post-exposure observation period.

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